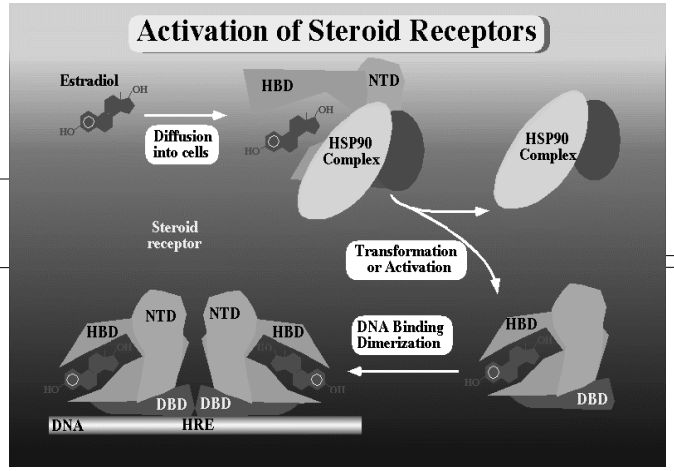
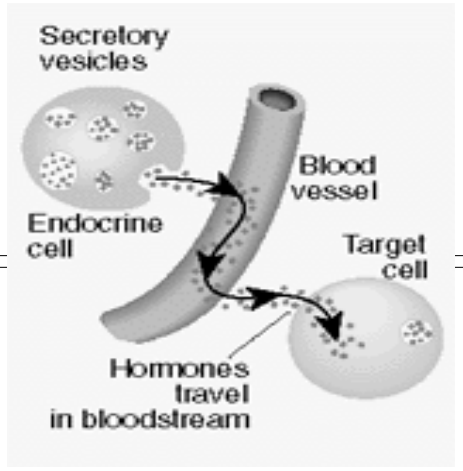
The Developmental Origins of Disease/Dysfunction : **Environmental Exposures and Epigenetic Mechanisms**





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National Institutes of Health, DHHS, USA

Thanks to those who contributed data…

- Shuk Mei Ho, University of Cincinnati
- Retha Newbold, NIEHS
- Mike Skinner, Washington State
- Ana Soto, Tufts University
- Moshe Szyf, McGill University
- Cheryl Walker, MD Anderson
- Fred vom Saal, University of Missouri

Overview

- Environment and Disease
- Epigenetics
- Developmental Basis of Disease
  - Fibroids
  - Breast cancer
  - Obesity
  - Fertility
  - Behavior
- Summary

Concept

All complex diseases are the result of :

- Gene-Environment Interactions over Time!

- Recent "epidemics" of chronic diseases like diabetes, childhood asthma, ADHD, obesity… must be due to environmental, dietary and behavioral changes.

- Question: If environmental exposures play an important role in disease and dysfunction why has it been so difficult to find/characterize ?

- Answer: We have been looking in the wrong place (wrong time), and with imprecise measurements of exposure.

It is also very complex!

Why is it so difficult to define the role of environment in disease in humans?

- **Expect effects to be small—mostly functional changes with some specific birth defects...requiring sensitive and specific endpoints.**
- **Expect effects to be difficult to detect due to human genomic variability and SNPs….requiring a genomic approach.**
- **Expect effects to be due to multiple chemicals with varying sensitivities and half lives… requiring a mixtures approach.**
- **Expect effects to be due to "multiple hits"… requiring a lifespan approach.**
- **Expect in utero exposure to be most sensitive…. requiring a developmental approach.**
- **Expect some effects to be trans-generational… requiring a multigenerational approach.**
- **Expect it to be difficult to prove. …impossible with current technology for exposure assessment and biomarkers of toxicity… requiring improved exposure assessment and biomarkers of exposure and toxicity.**

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Epigenetic Alterations: The Molecular "Imprint" Made by Developmental Programming

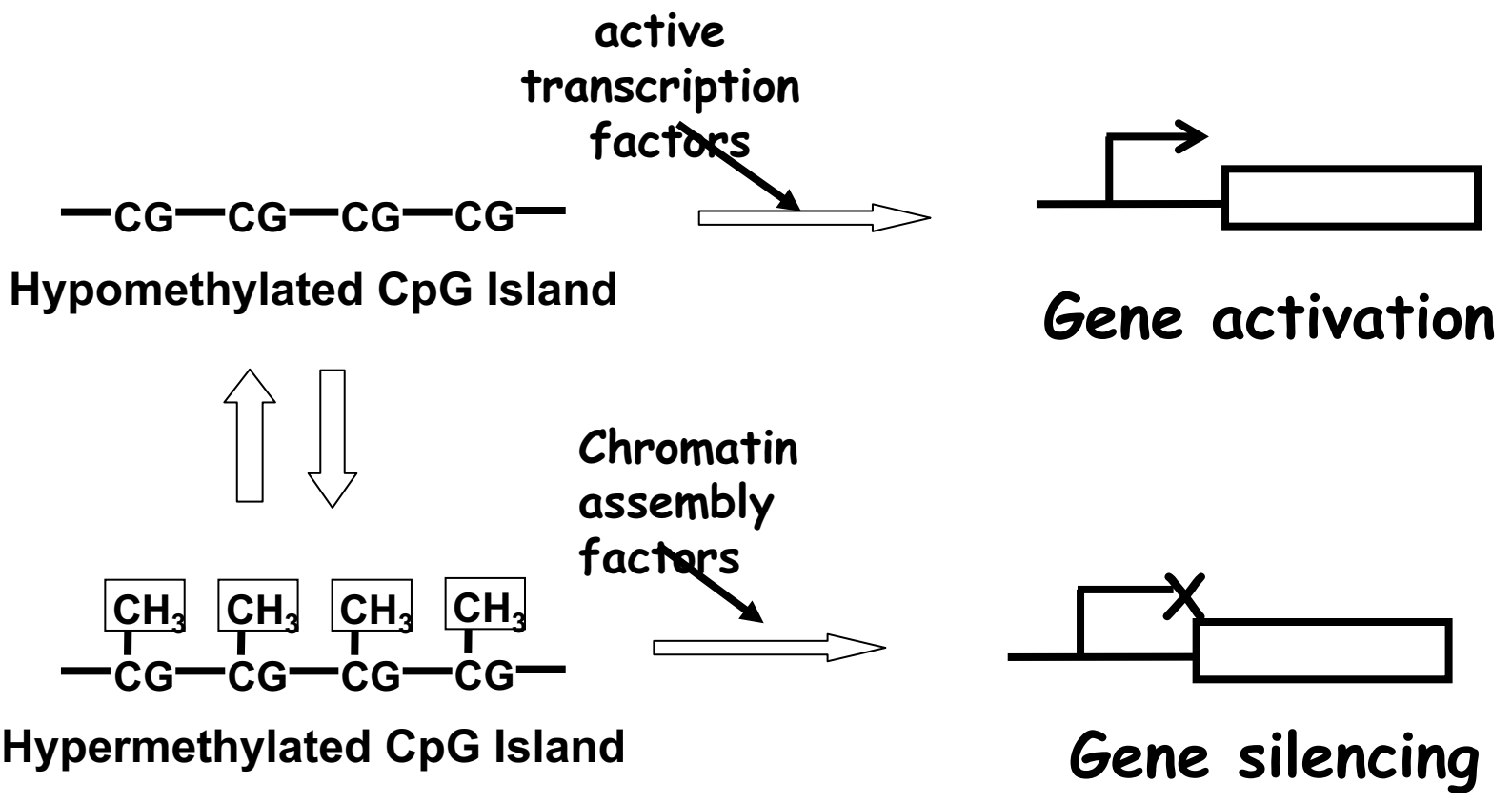
- Epigenetics… alterations that result in heritable changes in gene expression that do not involve changes in the DNA sequence
- Two types of epigenetic information (marks):
  - **Cytosine methylation (DNA)**
  - **Histone modifications (Protein)**
- Epigenetic marks determines the accessibility of the transcription machinery, which transcribes genes into mRNA
- Epigenetic marks control gene expression…on or off
- Epigenetic marks are set during development

DNA Methylation

- **CpG islands**
  - **1-2% genome**
  - **Non random**
  - **70% Promoter region, first or second exons and first intron**
  - **Inverse relationship between extent methylation and gene transcription**
  - **Methylation pattern sculpted during development by DNMTs and demethylases**
  - **Stable mark…Diagnostic!**
  - **Hypothesis: methylation is dynamic …sensitive to changes throughout life**

DNA Methylation

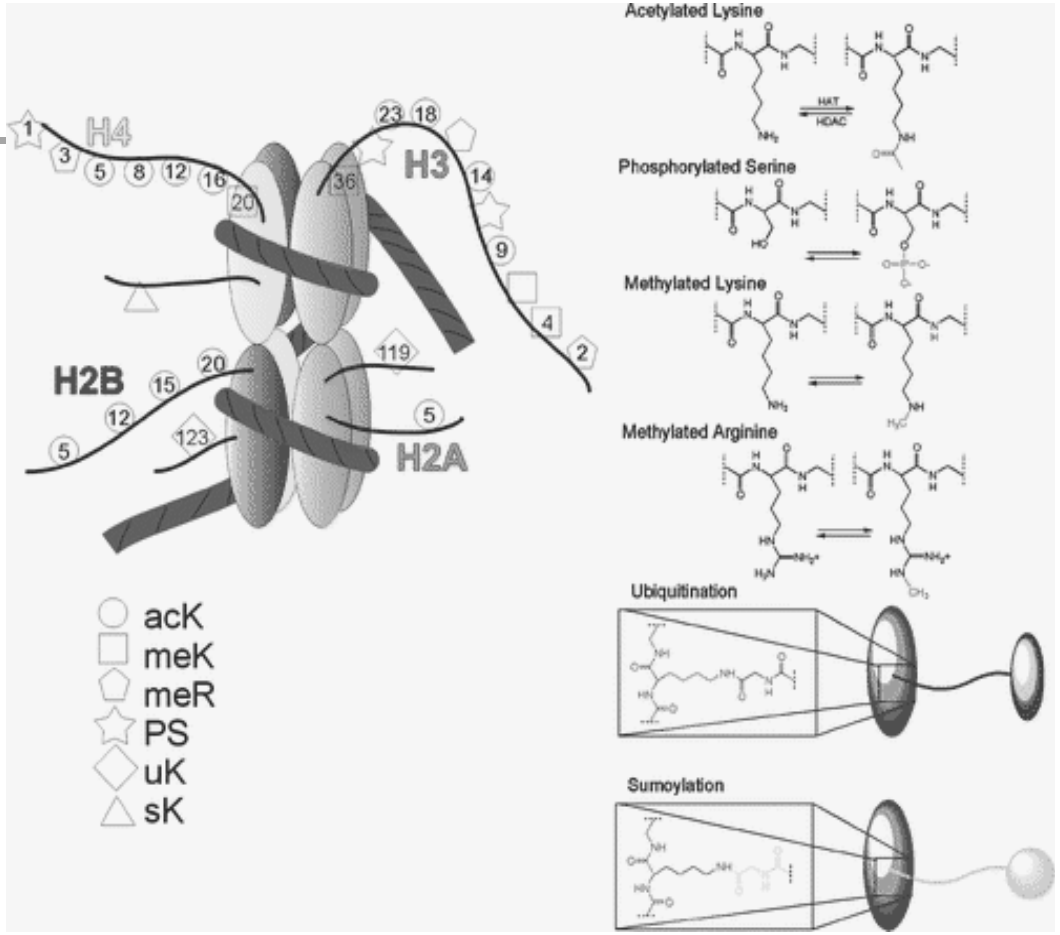
Reversible gene regulation by DNA methylation



Epigenetic mechanisms of Gene Regulation: Histone Modification:

•N-terminal tails of histones, are subject to various covalent modifications : *acetylation, methylation, phosphorylation ubiquitination.*

• Enzymes including
• histone deacetylase (HDAC), histone acetyltransferasease (HAT),
• histone methyltransferase (HMTase) are involved.



Daryl C. Drummond *et al*, 2004
Annual Review of Pharmacology and Toxicology
Vol. 45: 495-528

UNIVERSITY OF Cincinnati

Cancer Research Group

Environmental factors **(Chemicals, diet, drugs, stress, behavior)**

Epigenetics (stable but plastic)

Genetic polymorphisms (born with)

Inter-individual variability

Susceptibility to Disease, Toxicants, Drugs, Altered behavior

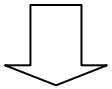
Shuk mei Ho

Developmental Exposures Alter Responses Later in Life

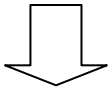
Diet, Maternal care, Drugs, Toxicants

Epigenetic Machinery Modulation

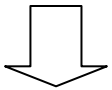
Inter-individual Epigenetic Variation



Altered Gene Expression Programming



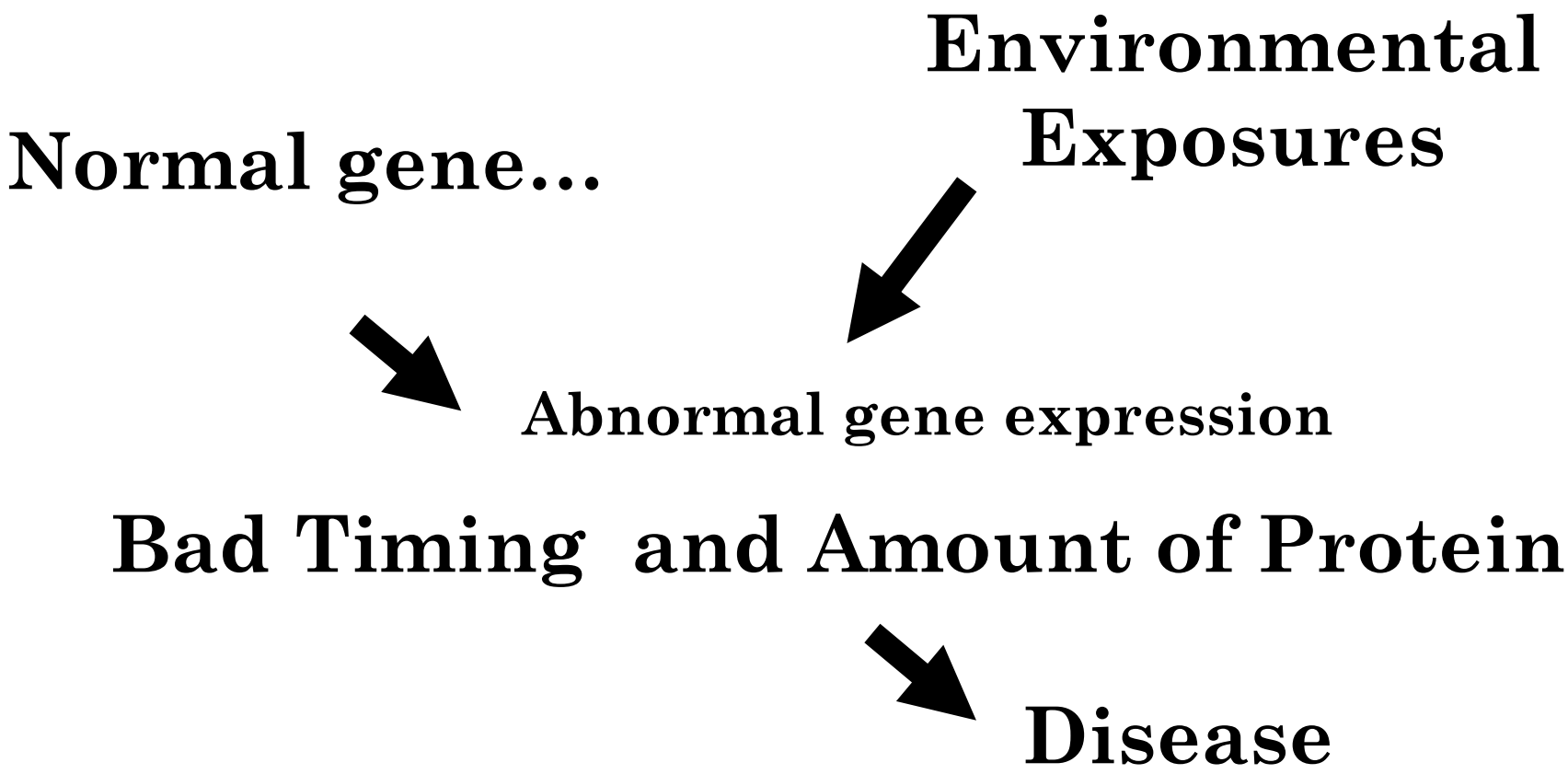
Phenotypic Variation



Susceptibility to Disease, Behavior, Sensitivity to Drugs

Szyf tox sci 2007

Epigenetic Basis of Disease!



Pete Myers

Epigenetic Basis of Disease!

Environmental

Norm

Environmental exposures acutely and directly cause altered gene expression via signal transduction pathways and alter the long-term timing of gene expression via epigenetics.

Bad Timing and Amount of Protein

Disease

Pete Myers

Concept

The epigenome is sensitive to and responds to environmental insults during development and throughout life. Development is the most sensitive period.

Epigenetics is a biological mechanism that allows the genome to adapt the to altered environments throughout life.

Epigenetic marks are heritable providing a mechanism for environmental-directed evolution.

Moshe Szyf

"Agents" Shown to Modify the Epigenome

- Methoxyclor
- Vinclozolin
- DES
- Bisphenol A
- Dioxin
- Cigarette Smoke
- Phytoestrogens
- Heavy metals
- Social environment
- High fat diet
- Modulation of one carbon metabolism (SAM/folic acid)
- Valproic Acid (HDAC inhibitor)
- Phenobarbital

Overview

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- Epigenetics
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Fetal Origin of Adult Disease: The Barker Hypothesis

- 1989 David Barker found an inverse relationship between birthweight and death from heart disease in England and Wales.
- Studies confirmed by "Dutch Hunger Winter" when food supplies to occupied Netherlands were cut off by Nazis. Individuals born during this time had high incidence as adults of insulin-resistance.

Fetal Origin of Adult Disease (FEBAD) confirmed for

Coronary heart disease

Hypertension

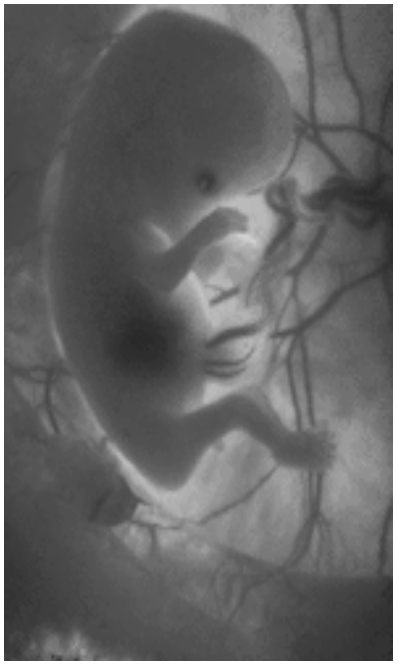
Type II diabetes

Table 1. Hazard ratios for coronary heart disease according to body size at birth[a]

	Hazard ratio (95% CI)	No. of cases/No. of men
Birthweight (g)		
<2500	3.63 (2.02–6.51)	24/160
–3000	1.83 (1.09–3.07)	45/599
–3500	1.99 (1.26–3.15)	144/1775
–4000	2.08 (1.31–3.31)	123/1558
>4000	1.00	21/538
P for trend	0.006	
Ponderal index (kg m^{-3})		
<25	1.66 (1.11–2.48)	104/1093
–27	1.44 (0.97–2.13)	135/1643
–29	1.18 (0.78–1.78)	84/1260
>29	1.00	31/578
P for trend	0.0006	

Cheryl Walker

Concept



- Fetus in utero responds to environmental cues: nutrition, stress.
- Depending on in utero conditions it prepares for life….under the assumption that life after birth will match the conditions in utero.
- A mismatch leads to increased susceptibility to disease.

Why is the developmental period super sensitive to environmental chemicals? "The Fragile Fetus"

- The developing organism (fetus and neonate) is extremely sensitive to perturbation by chemicals because….

  - Tissues/organs forming
  - Lack of DNA repair
  - No Immune system
  - No Blood/brain barrier
  - Immature Detox enzymes
  - Poor Liver metabolism
  - Epigenetic marks set

Why is the developmental period super sensitive to environmental chemicals? "The Fragile Fetus"

- **The developing organism (fetus and neonate) is extremely sensitive to perturbation by chemicals because….**

Organ development proceeds via an intricately orchestrated, temporal pattern of gene expression that is specific to the developing tissue. As a result, toxic exposures that perturb gene expression may have unique effects in the developing tissue or organ.

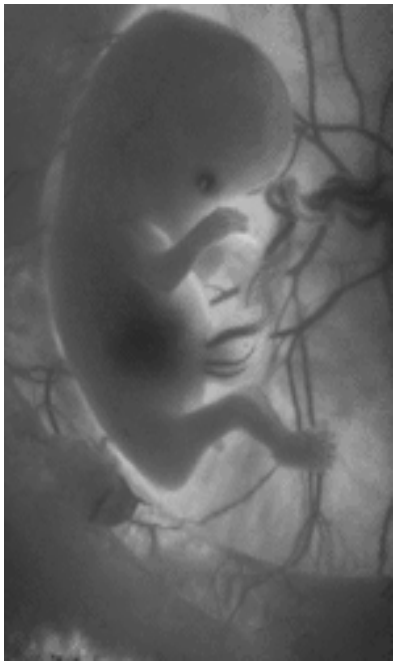
  - **Poor Liver metabolism**
  - **Epigenetic marks set**

Developmental Exposures to Environmental Chemicals

- **Teratology**
  - Death
  - Birth Defects
  - Low Birth Weight
  - Functional Changes

  Many chemicals will cause all effects depending on the timing and dose!

Concept



- There is no doubt that development is the most sensitive time for environmental exposures…in animals and humans.
- Exposure to children is higher than adults.
- Low environmentally relevant exposures during development cause “functional changes”.

Developmental Basis of Disease: Disease Focus in Animals

- Reproductive/Endocrine
  - Breast/prostate cancer
  - Endometriosis
  - Polycystic ovary syndrome
  - Fertility
  - Diabetes/metabolic syndrome
  - Puberty
  - Obesity
- Brain/Nervous System
  - Alzheimer's disease
  - Parkinson's disease
  - ADHD
- Pulmonocardiovascular
  - Atherosclerosis
  - Asthma
  - Chronic obstructive pulmonary disease
  - Heart disease/hypertension
- Immune/Autoimmune
  - Systemic/tissue specific autoimmune disease
  - Immunosuppression

Developmental Basis of Disease: Environmental Stressor Focus

- Environmental Estrogens
  - Diethylstilbestrol
  - Genistein
  - Bisphenol A
- Tributyl Tin
- Phthalates
- Dioxin/PCBs
- Atrazine
- Smoking/ETS/ Air Pollution
- Methylmercury/Lead/arsenic
- LPS
- Vinclozolin
- Polybrominated diphenyl ethers (PBDE)

NON Mutagenic Effects

Developmental Basis of Disease: Examples

- **Animal Studies**
  - Fibroids
  - Breast cancer
  - Obesity
  - Fertility
  - Behavior

Thought Provoking Idea!

Could it be that susceptibility to

uterine fibroids

is determined during development and by environmental exposures?

Developmental Basis of Adult Disease:
DES as Proof of Principle (Retha Newbold, NIEHS)

A DES Ad from 1957

- **Diethylstilbestrol (DES), a synthetic estrogen, was synthesized by Sir Edward Charles Dodds in 1938.**
- **DES was widely prescribed from the 1940s thru the 1970s for the treatment of threatened miscarriage.**
- **Considered safe and effective, also prescribed for normal pregnancies.**
- **Total # treated pregnancies unknown; worldwide estimates ~ 2-8 million.**
- **Adverse effects are now well known;**
  - **Low incidence of vaginal cancer in female offspring.**
  - **High incidence of reproductive tract dysfunction (male & female offspring).**

Comparative Developmental Effects of Prenatal Exposure to DES in Mice and Humans

	Male Offspring	Female Offspring
Functional Changes	Subfertility/Infertility Decreased Sperm Counts	Subfertility/Infertility Poor Repro. Outcome
Birth Defects	Microphallus & Hypospadias Retained Hypoplastic Testes Retained Mullerian Remnants (anatomical feminization)	Oviduct, Uterus, Cervix and Vagina Paraovarian Cysts of Mesonephric Origin

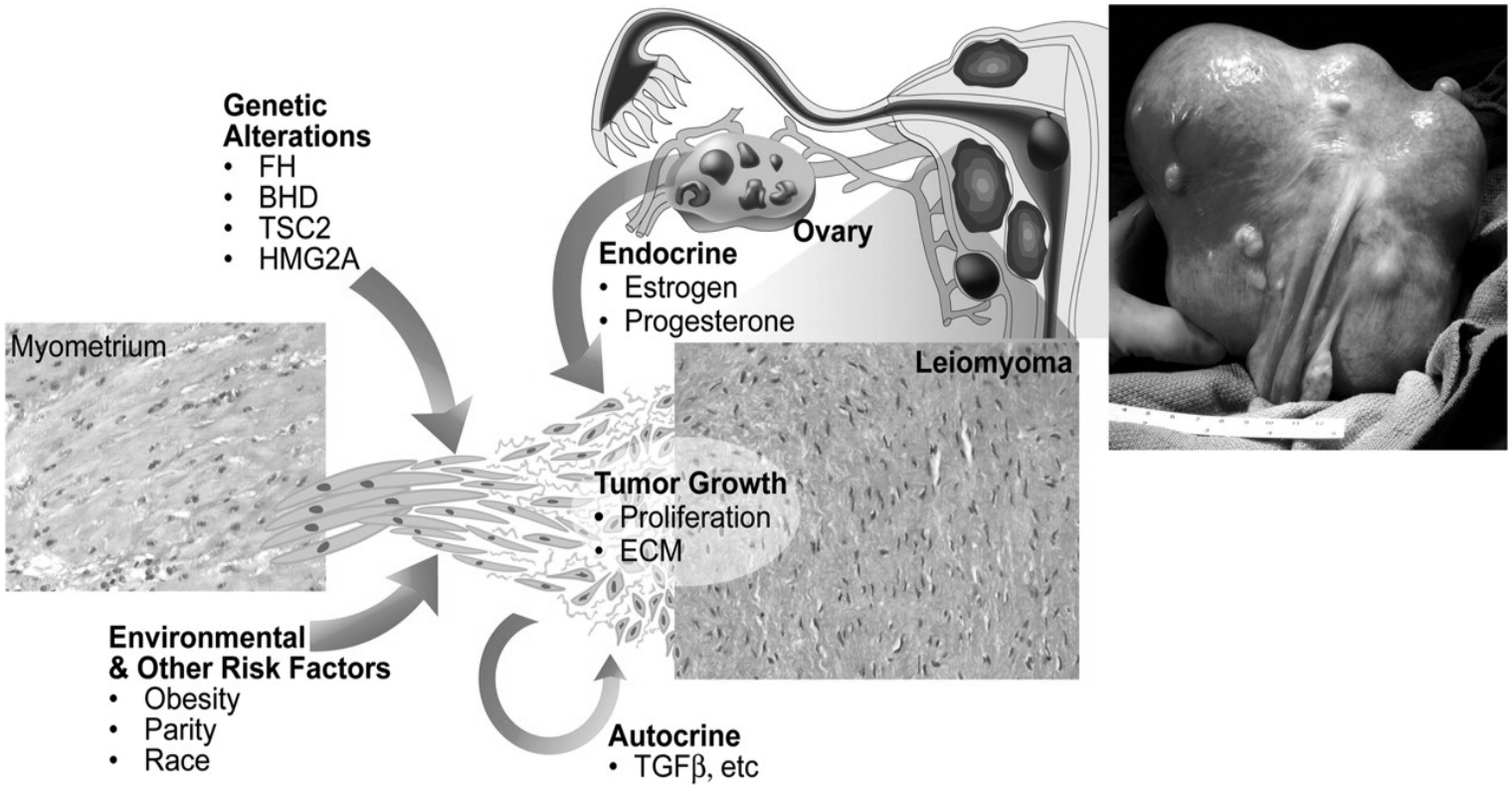
Animal studies mimic Human data

Tumors	Testicular Tumors Tumors in Retained Mullerian Remnants Epididymal Cysts Prostatic Tumors & Inflammation	Proliferative Epithelial Lesions in Oviduct Vaginal Adenomyosis & Adenocarcinoma

Newbold 1999

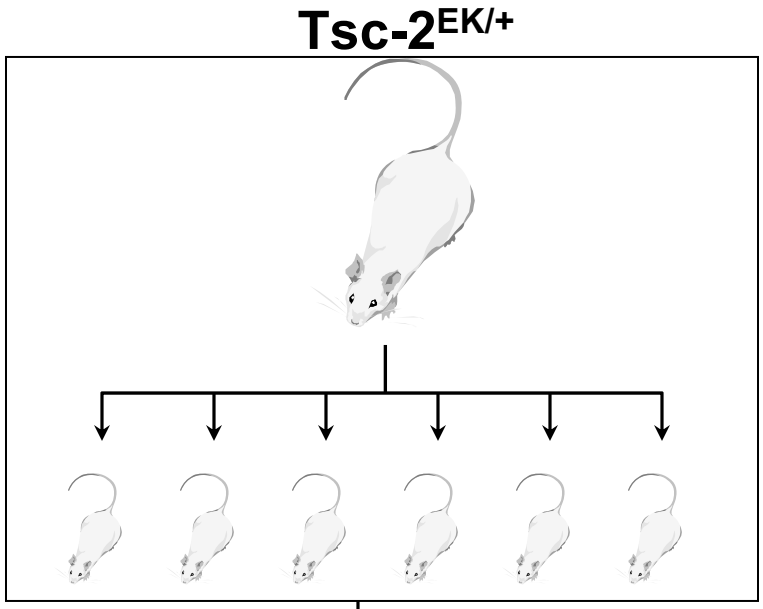
Uterine Leiomyoma

Walker and Stewart
Science 2005



- Most common tumor of women
- Number 1 indication for hysterectomy in the US, accounting for >2000,000 of these surgeries annually
- Hormone dependent requiring estrogen for growth (Cheryl Walker)

The Developmental Basis of Uterine Leiomyoma: Role of Tumor Suppressor Gene Penetrance



- Tumor: Uterine Leiomyoma
- Tumor Suppressor Gene: TSC2
- Model: Eker rat
- Environmental Agent: Exposure to the xenoestrogen DES

♀ Inject with 10µg DES or vehicle Postnatal days 3, 4, 5

Sacrifice 5 mos, 16 mos.

- Carrier (Tsc-2$^{EK/+}$) + DES
- Carrier (Tsc-2$^{EK/+}$) + Vehicle
- Wildtype (Tsc-2$^{+/+}$) + DES
- Wildtype (Tsc-2$^{+/+}$) + Vehicle

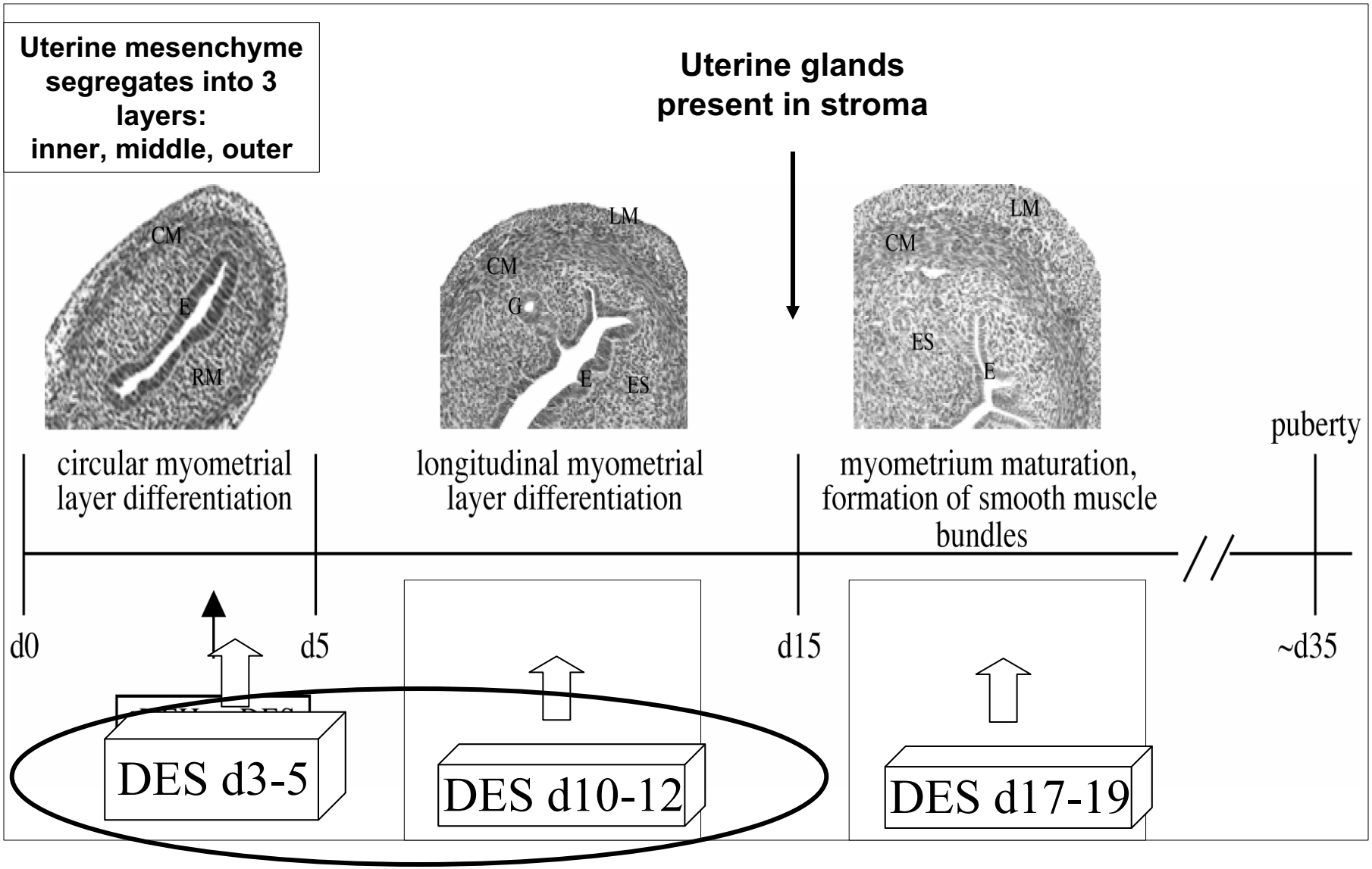
Developmental DES Exposure Increases Tumor Incidence, Multiplicity and Size in Genetically Susceptible Animals.

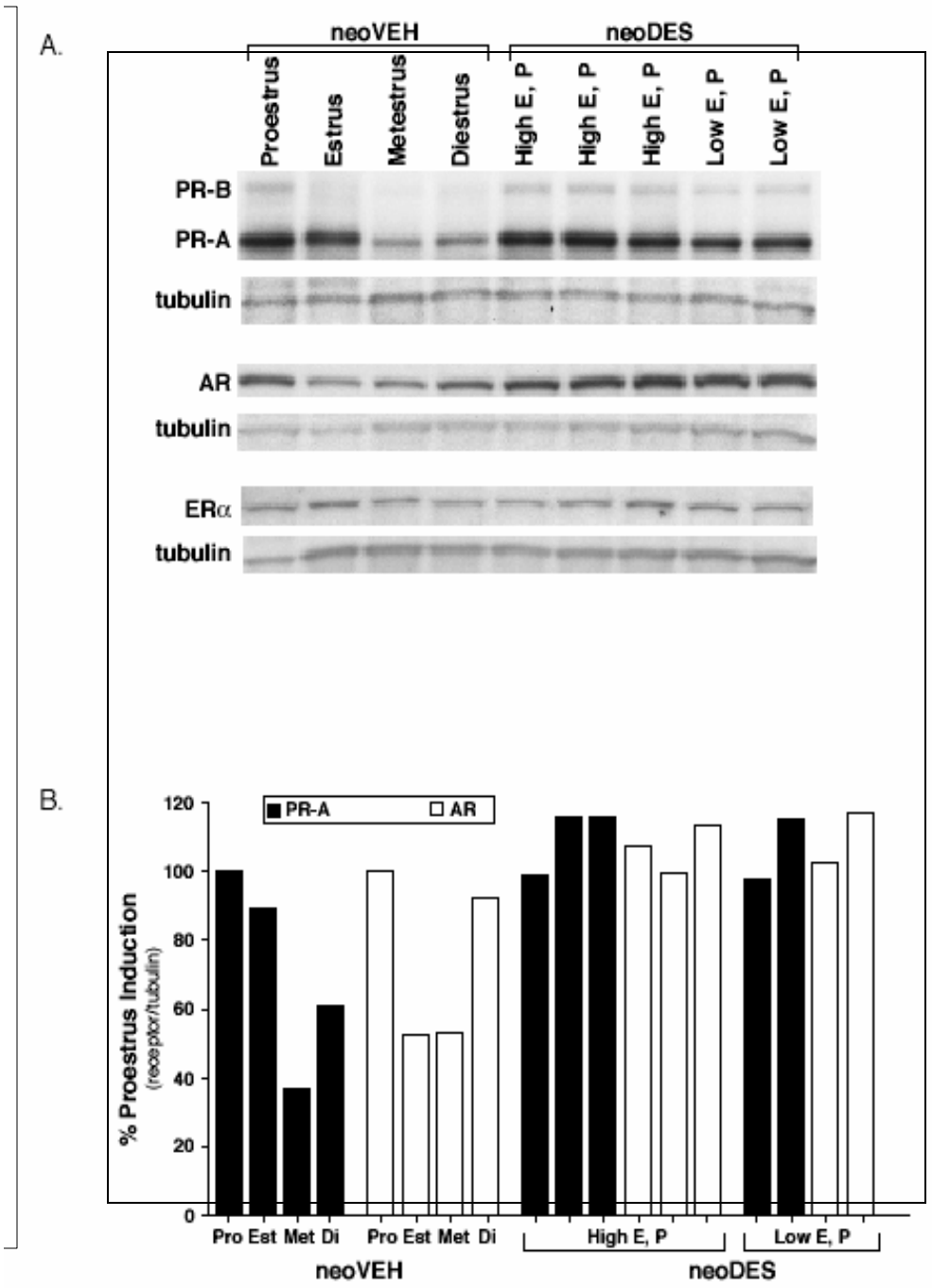
Genotype	Treatment	N of rats	% Tumor Incidence	Multiplicity (mean no. of tumors/rat)	Size (cm^3) Mean ± S.E.M.
Tsc-2$^{Ek/+}$	vehicle	28	64	0.82	2.3 ± 1.1
	DES	24	92*	1.33*	10.5 ± 2.7*
Tsc-2$^{+/+}$	vehicle	34	0	N/A	N/A
	DES	34	0	N/A	N/A

Developmental reprogramming of estrogen responsiveness

Cook et al. PNAS 2005

Window of Susceptibility to Developmental Programming: When does it Close?

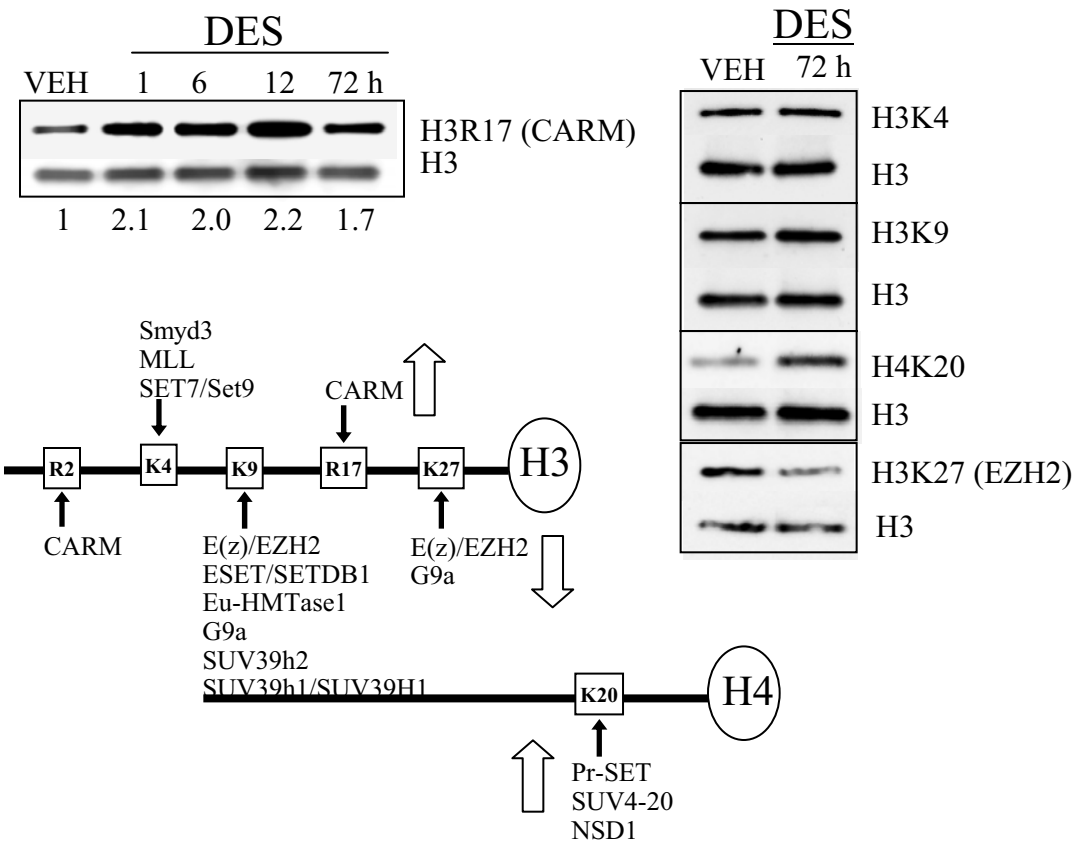




Developmental Re-programming of Estrogen Responsiveness in DES Females

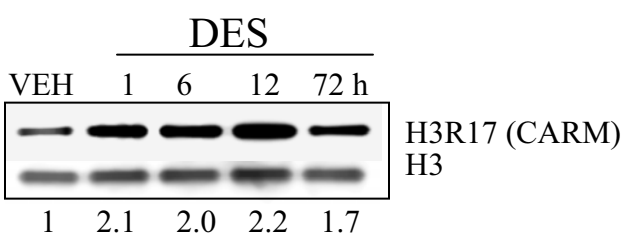
- Target myometrial cells in DES animals hyper-responsive to (low) estrogen levels
- Not observed in liver, which is fully developed in neonates
- Estrogen receptor levels unchanged
- **Developmental exposure had reprogrammed estrogen responsiveness**

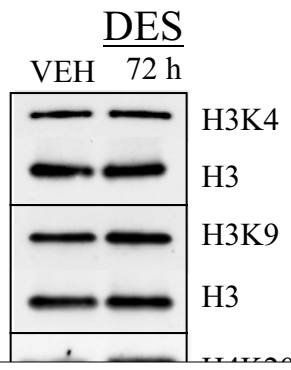
DES Modulates Histone Methylation in Neonatal Uteri



- DES induces global changes in histone methyl marks
- CARM1, an ER coactivator and histone methyltransferase, methyl mark (H3R17) increases ⇧
- EZH2, a methyltransferase inhibited by AKT, methyl mark (H3K27) decreases ⇩

DES Modulates Histone Methylation in Neonatal Uteri





- DES induces global changes in histone methyl marks
- CARM1, an ER

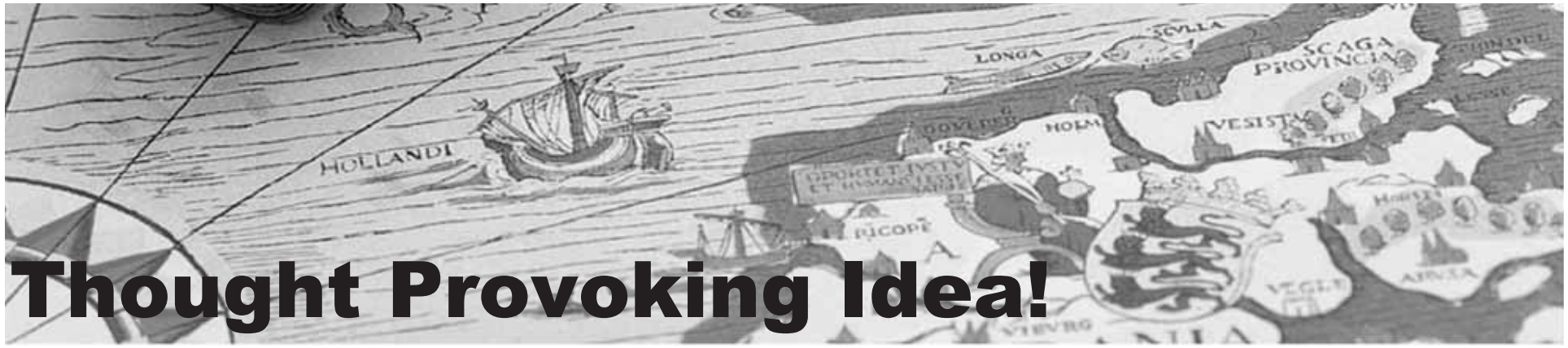
Identification of an “imprint” left by developmental programming such as altered methyl marks may be useful for identification of exposed individuals and as a biomarker for disease susceptibility in adult life

Smyd3
MLL
PI-SET
SUV4-20
NSD1

methyltransferase inhibited by AKT, methyl mark (H3K27) decreases ⇩

Concept

- Environmental agents act on a genetic background.
- Environmental exposures can act synergistically with genetic susceptibility factors, in critical pathways to increase susceptibility to disease.
- Developmental exposures leave epigenetic marks….



Thought Provoking Idea!

Could it be that other major
reproductive diseases
endometriosis
premature menopause
PCOS
have their origins in development
and are influenced by
environmental exposures?

Thought Provoking Idea!

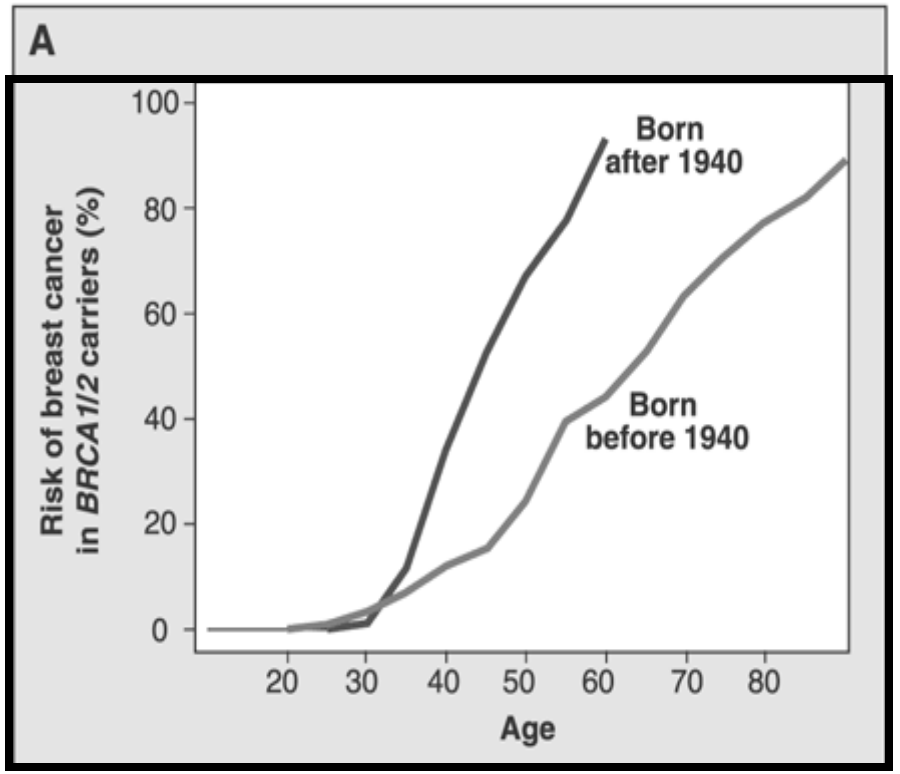
Could it be that

Breast cancer susceptibility

is determined during development and influenced by environmental exposures?

Gene-Environment Interactions Influence Cancer Risk

- Increased lifetime risk of breast cancer in women born after 1940
- Increased exposure to environmental estrogens
  - Phytoestrogens
  - Oral contraceptives
  - Pesticides
  - plasticizers

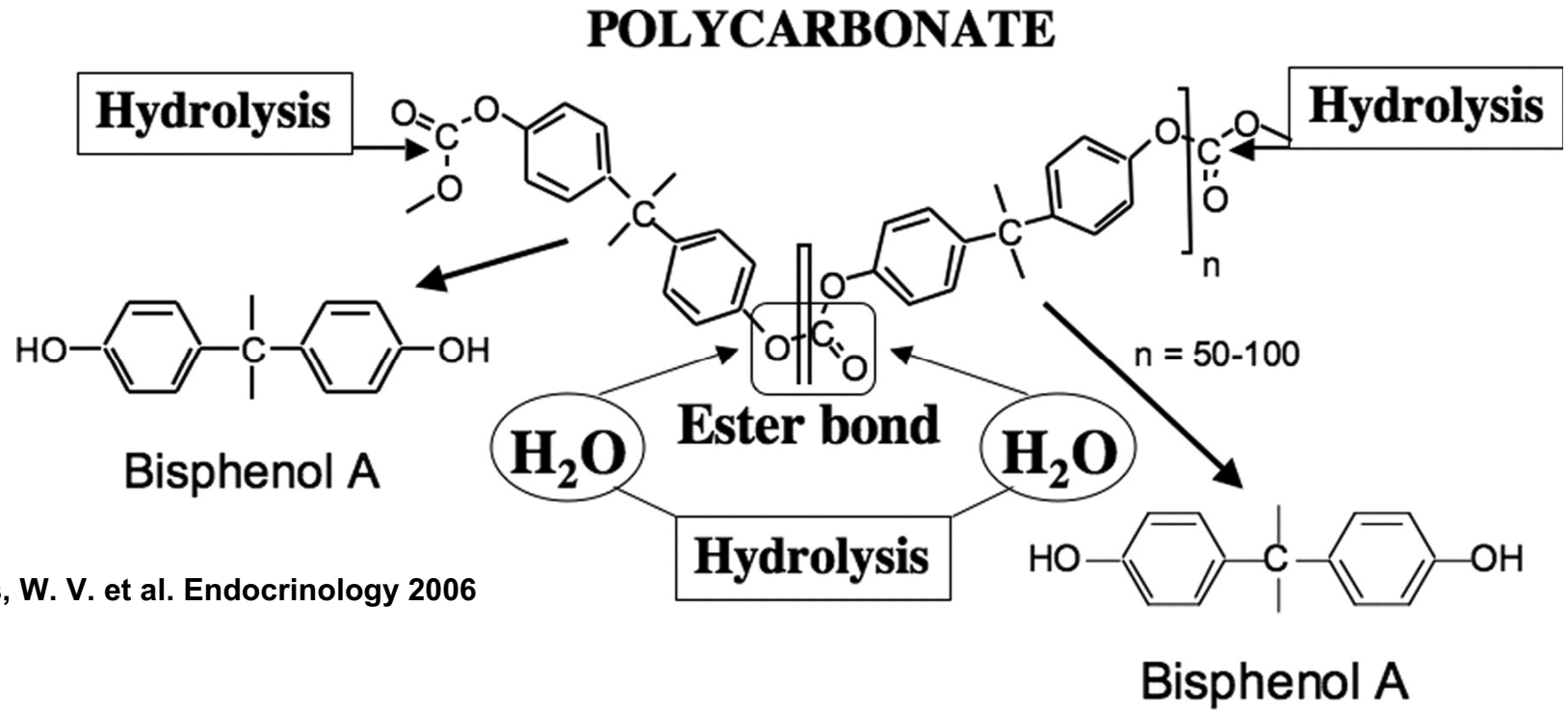


Importance of Environmental Factors on Cancer Risk in BRCA1/2 Ashkenazi Jew mutation carriers

***Science*, October 2003**

USES OF BISPHENOL A IN PRODUCTS
Production Capacity > 6.5 Billion Pounds / Year

BISPHENOL-A



Welshons, W. V. et al. Endocrinology 2006

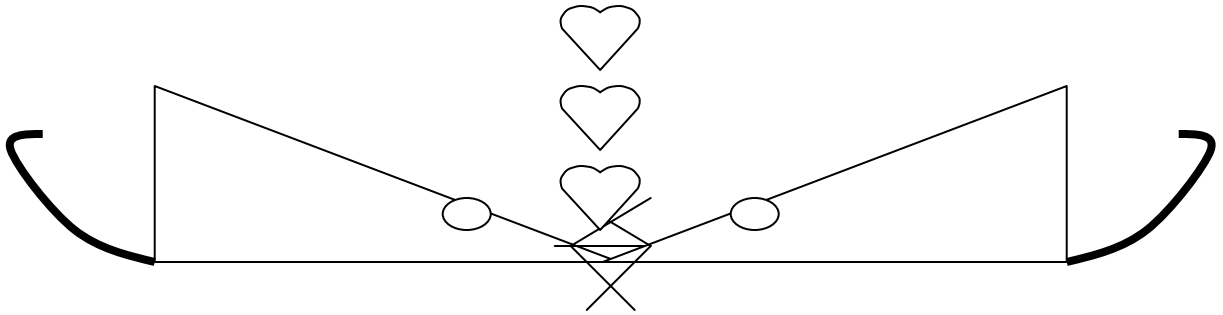
Bisphenol A-based polycarbonate is used as:

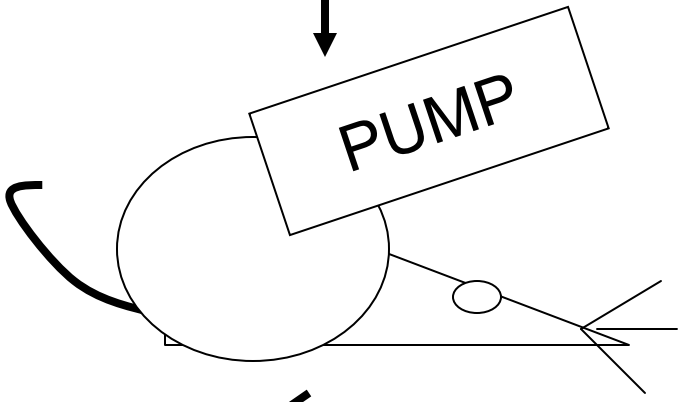
- a plastic coating for children's teeth to prevent cavities
- as a coating in metal cans to prevent the metal from contact with food contents
- as the plastic in food containers, refrigerator shelving, baby bottles, water bottles, returnable containers for juice, milk and water, micro-wave / oven-ware and eating utensils.

PRENATAL-NEONATAL EXPOSURE OF MICE AND RATS TO BISPHENOL A AT HUMAN EXPOSURE LEVELS IN RELATION TO HUMAN HEALTH TRENDS

EFFECTS IN MICE & RATS	HUMAN HEALTH TRENDS
Abnormal urethra	Abnormal penis+urethra
Prostate hyperplasia & cancer	Prostate cancer increase
Mammary hyperplasia & cancer	Breast cancer increase
Sperm count decrease	Sperm count decrease
Early puberty in females	Early sexual maturation
Hyperactivity/Impaired learning	ADHD
Abnormal oocytes	Miscarriage*
Body weight increase	Obesity increase*

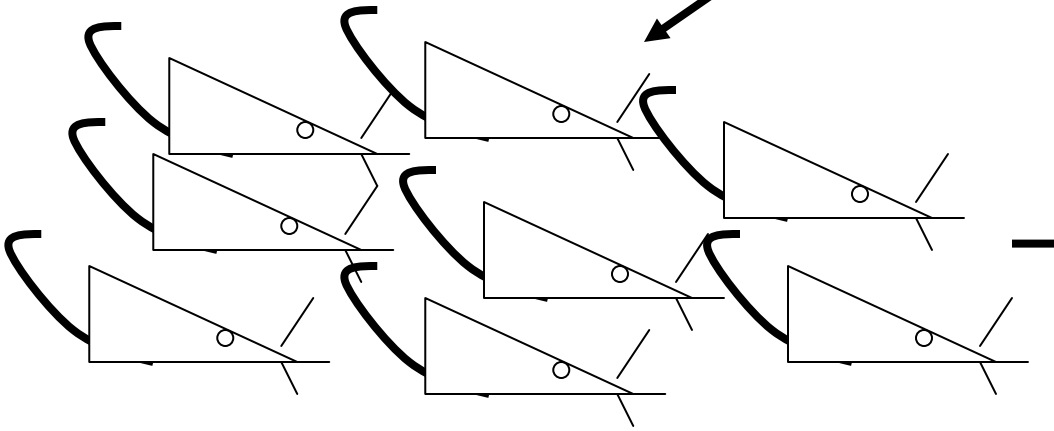
Fetal Basis of Breast Cancer: Bisphenol A Research Paradigm

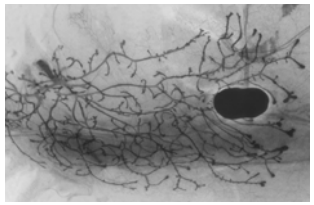




preg day 8- birth

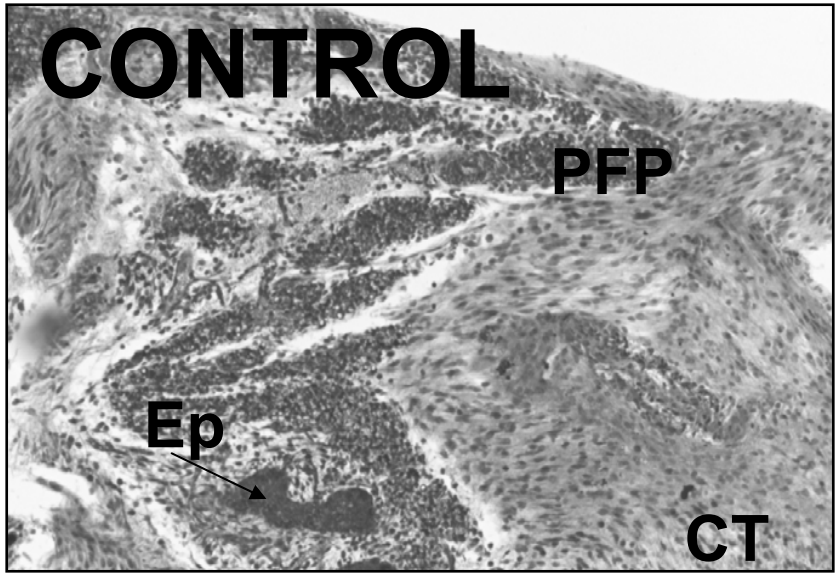
0, 25, 250, ng BPA/kg body weight/day

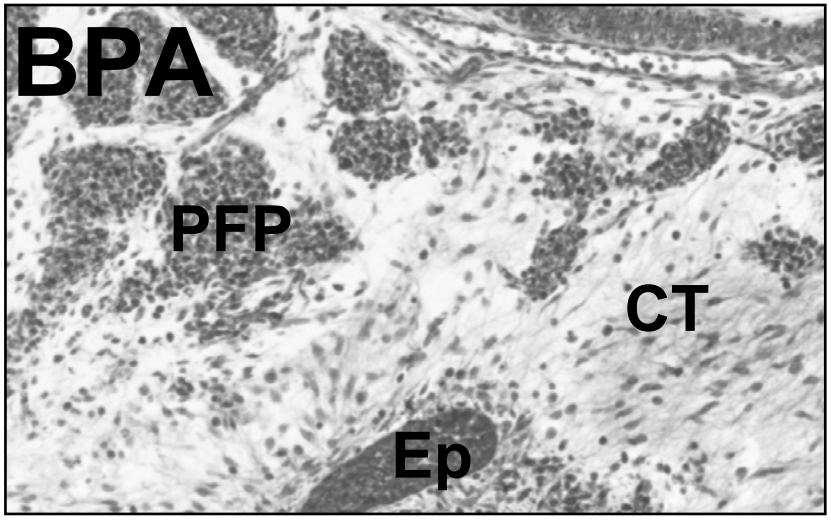




Ana Soto

Exposure to BPA alters overall organization of the fetal Mammary Gland





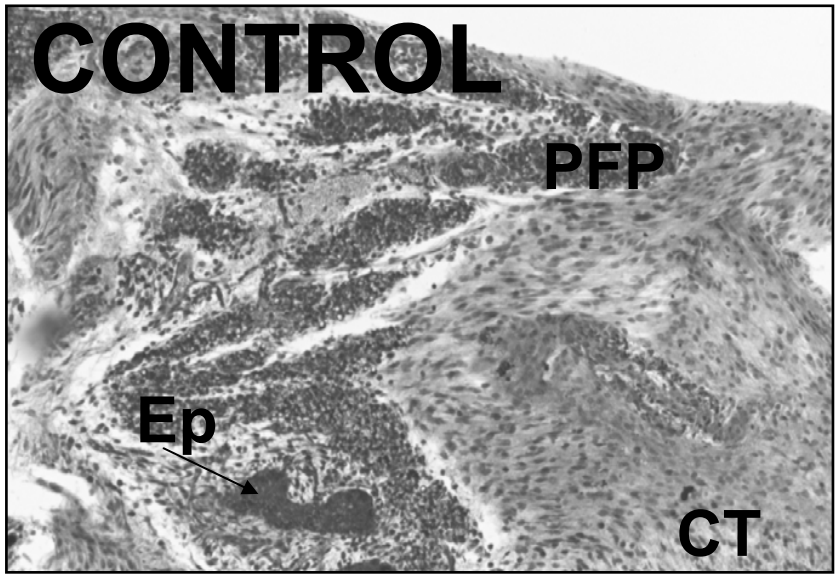
Accelerated maturation of the stroma, increased accumulation of fat droplets into fat pad adipocytes

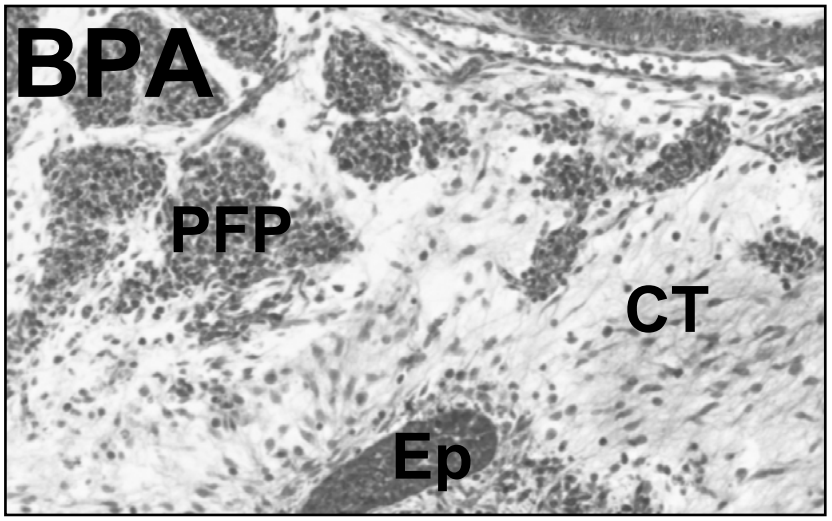
Increased number of terminal ends

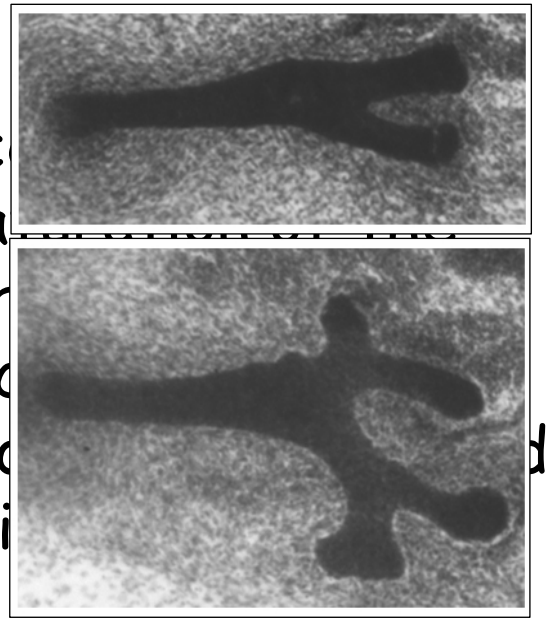
Increased area subtended by ducts

Increased ductal extension

Exposure to BPA alters overall organization of the fetal Mammary Gland





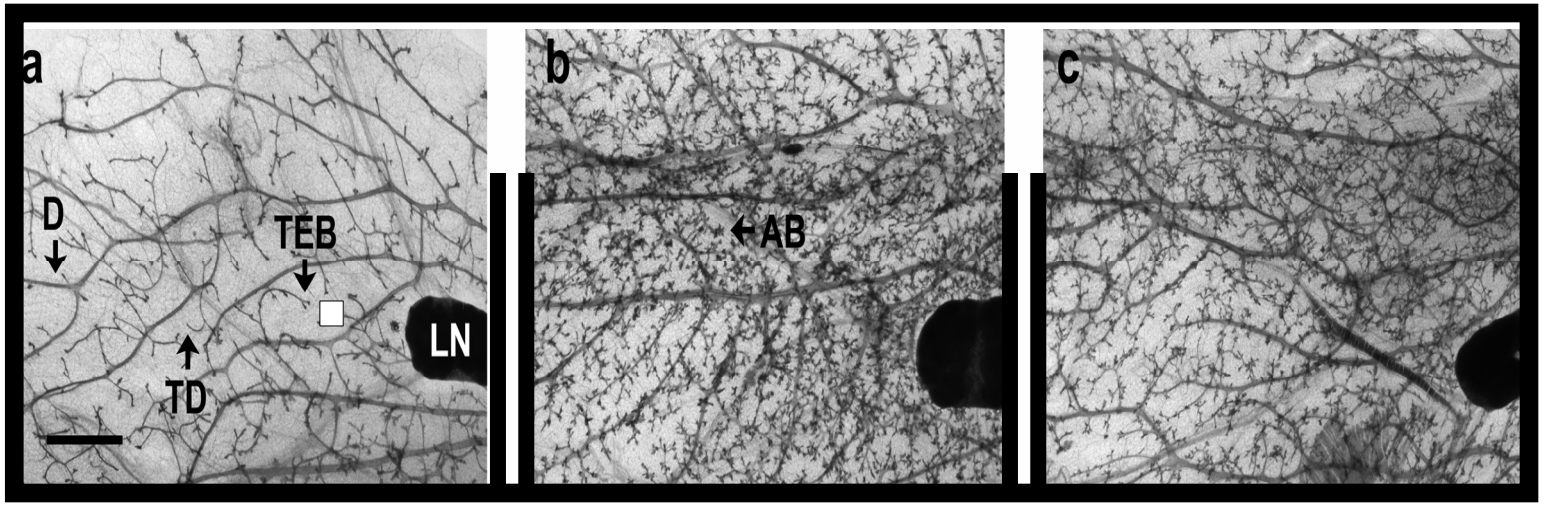


Increased number of terminal ends

Increased area subtended by ducts

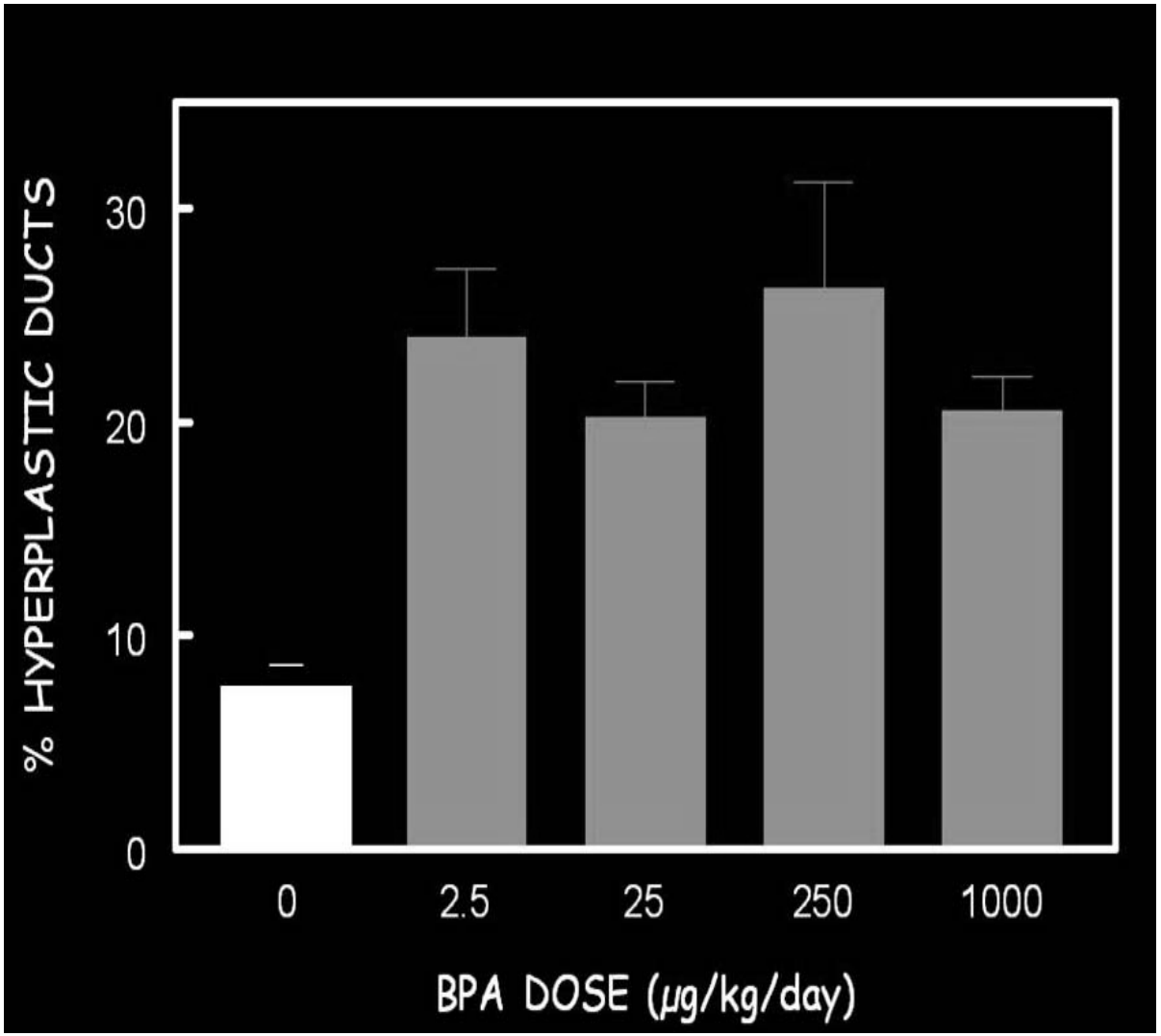
Increased ductal extension

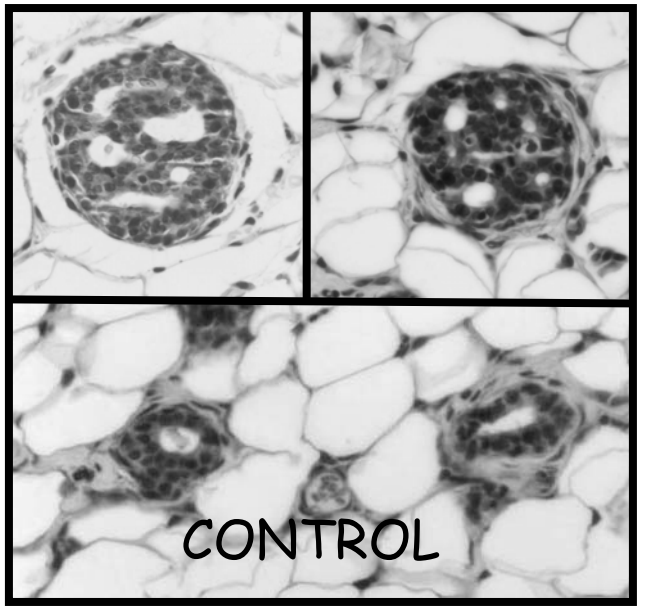
Mammary Gland Development: 6 Months



control 25 ng/kg BPA 250 ng/kg BPA

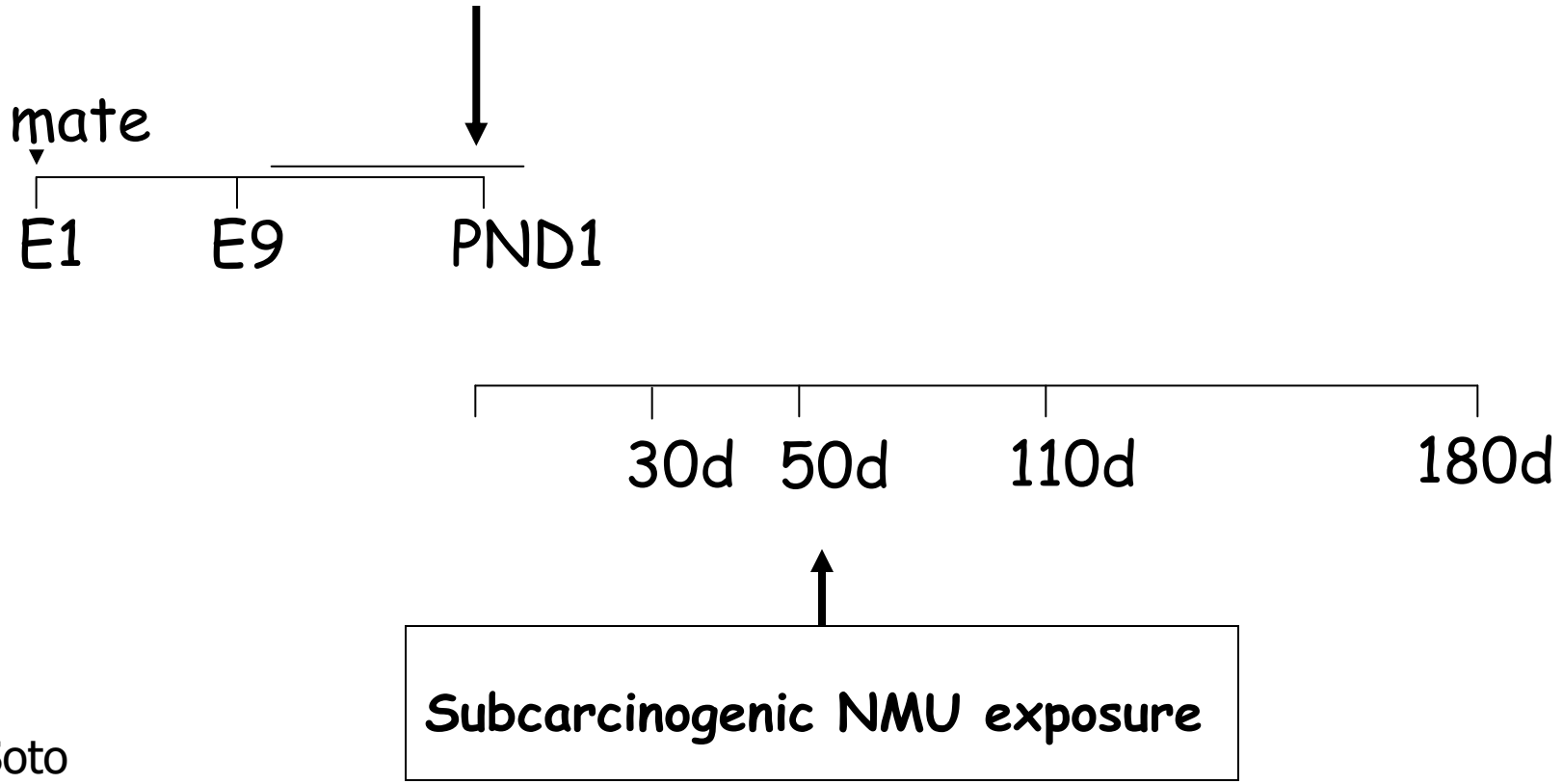
BPA Induces Ductal Hyperplastic Lesions and CIS





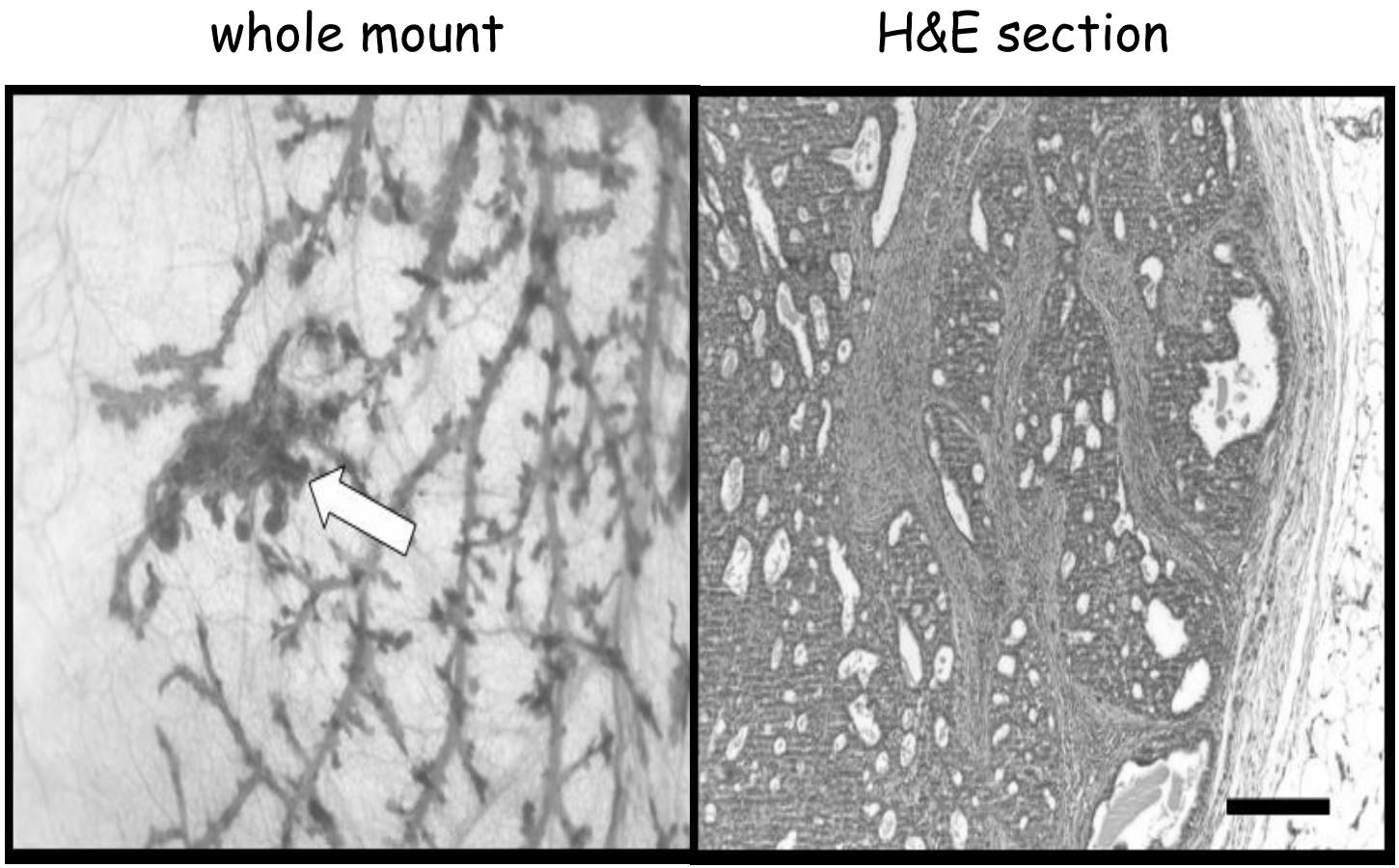
Experimental aim: To determine whether fetal exposure to BPA increases mammary cancer risk

0, 2.5, 25, 250, 500 and 1000 µg BPA/kg body weight/day



Ana Soto

BPA Increased the Incidence of Tumors After a Subcarcinogenic Dose of NMU



Concept

- Environmental exposures at environmental levels can cause cancer later in life.
  - Breast cancer
  - Prostate cancer
- In addition to developmental exposures sometimes additional exposures are needed.
  - First exposure sensitizes system to second

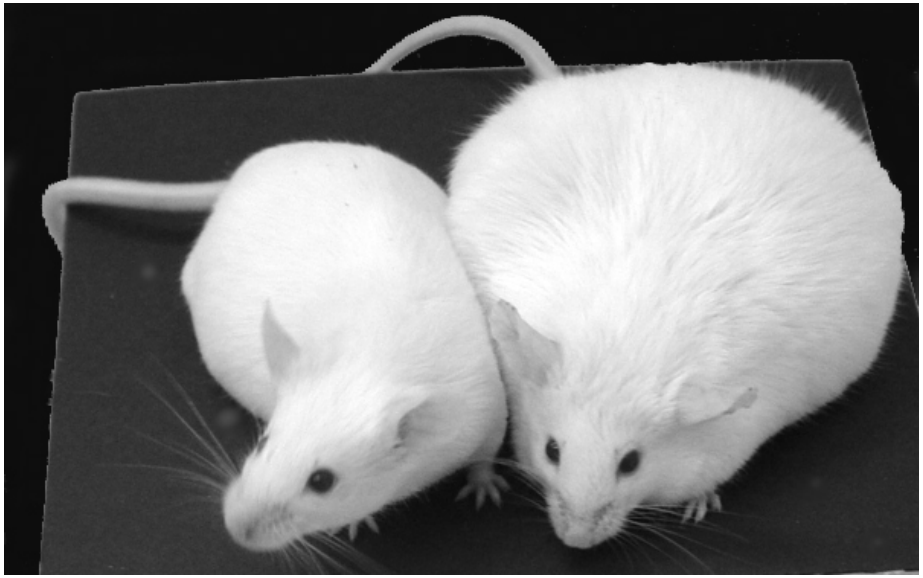
Thought Provoking Idea!

Could it be that

Obesity

is determined during development and influenced by environmental exposures?

Obesity: Lessons From Two Mice



Newbold *et al.* 2005, 2007

1 part per billion DES

100 ppb causes weight loss

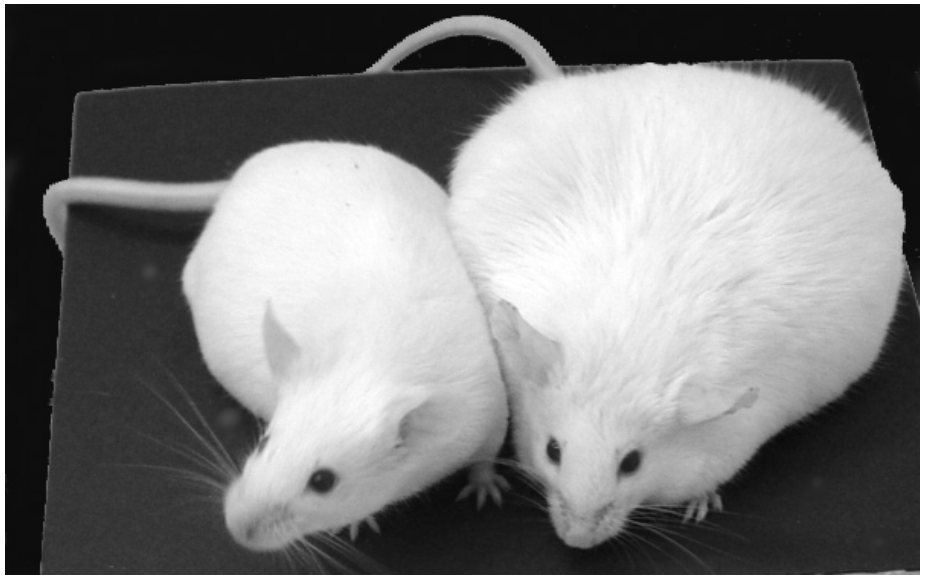
Exposure in the womb

--> Obese as adult

Same strain of mice
Same caloric intake
Same activity levels

Pete Myers

Obesity: Lessons From Two Mice



Newbold *et al*. 2005, 2007

1. Low levels matter

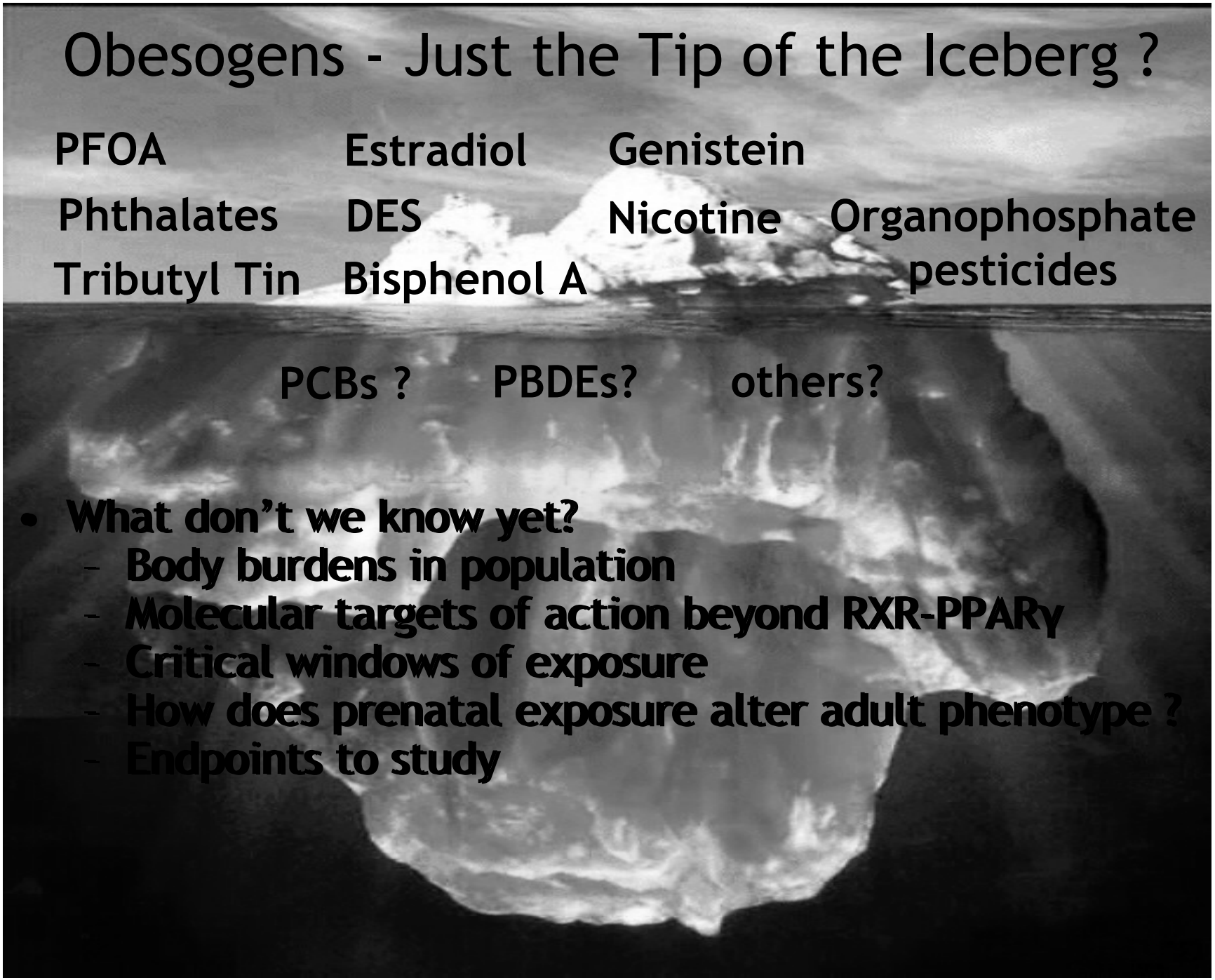
2. High level tests don't predict low level impacts

Same strain of mice
Same caloric intake
Same activity levels

3. Fetal exposures alter adult health

Pete Myers

Obesogens - Just the Tip of the Iceberg ?



- **What don't we know yet?**
  - **Body burdens in population**
  - **Molecular targets of action beyond RXR-PPARγ**
  - **Critical windows of exposure**
  - **How does prenatal exposure alter adult phenotype ?**
  - **Endpoints to study**

Thought Provoking Idea!

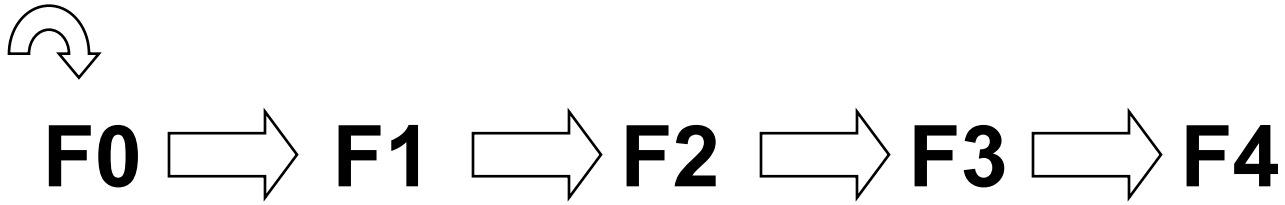
Could it be that

The effects of developmental exposure could be transmitted to future generations

and influence their adult sensitivity disease ?

Fetal Basis and Transgenerational Transmission of Reduced Fertility

Endocrine Disruptor



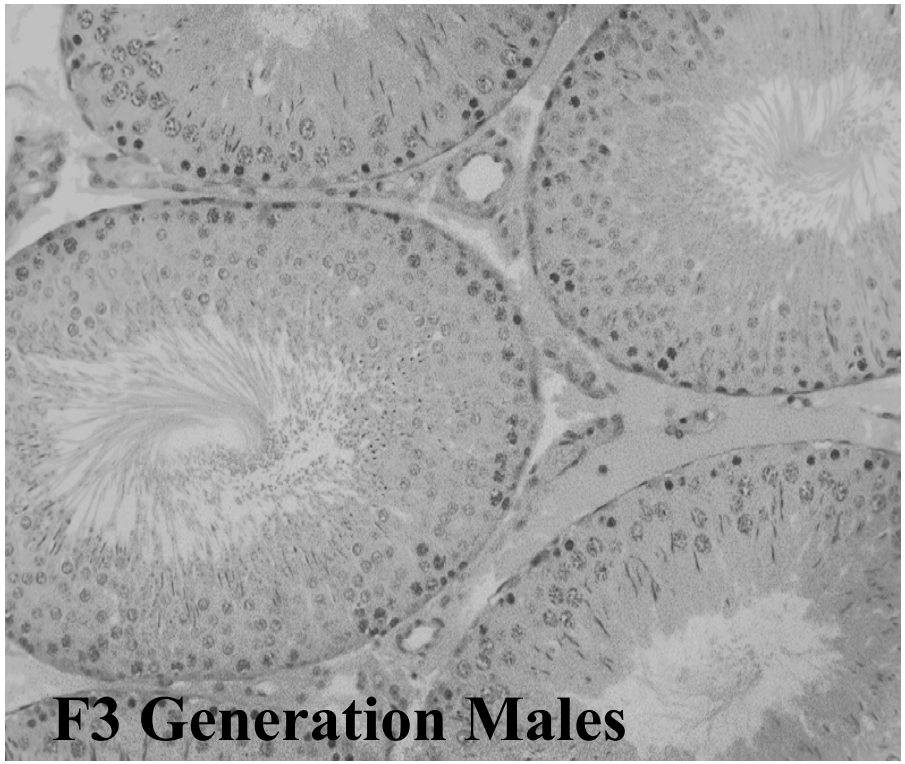
Vinclozolin Methoxychlor

Anway et al Science 308, 2005
Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility

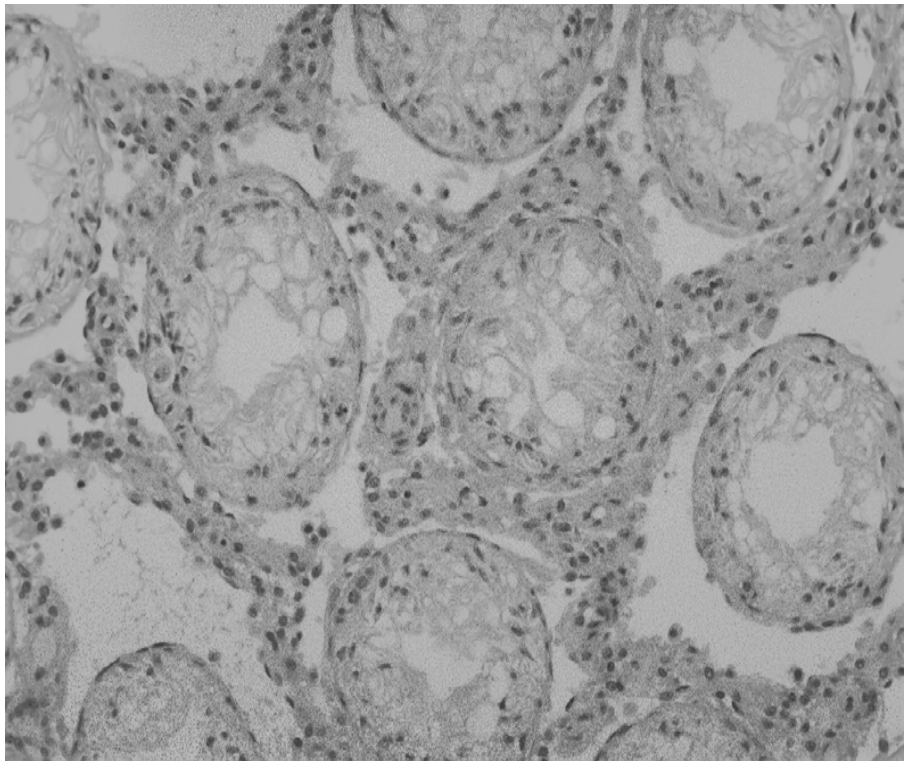
Developmental Exposure to vinclozolin and 3rd Generation Testicular Morphology

Complete Male Infertility (10%):
100% altered spermatogenesis

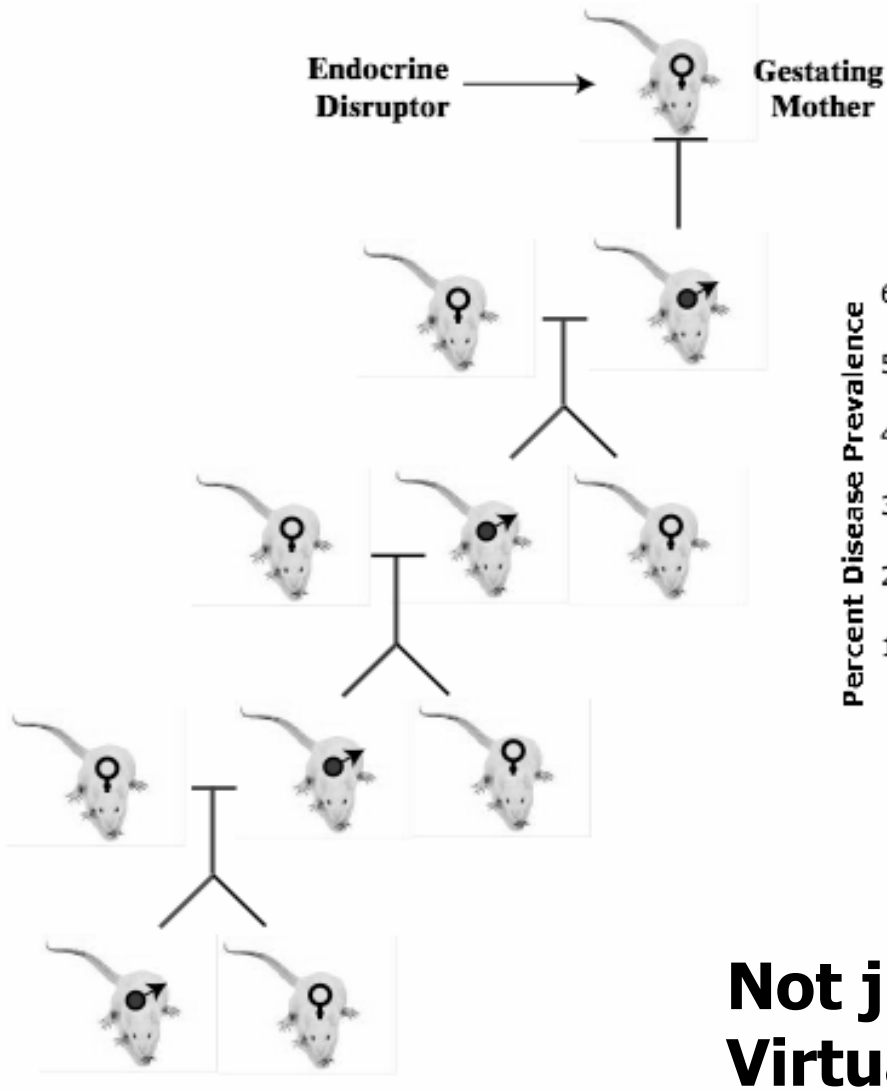
Control

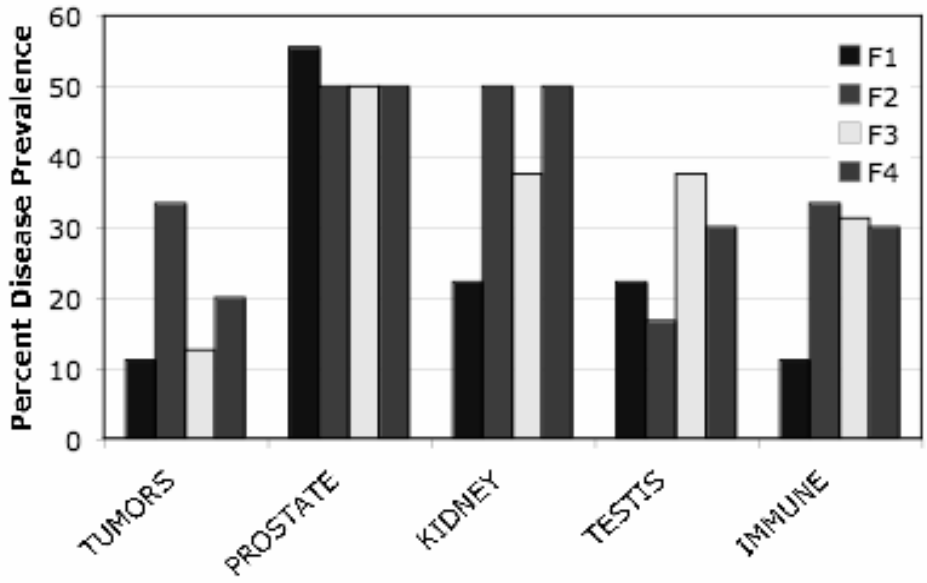


Vinclozolin



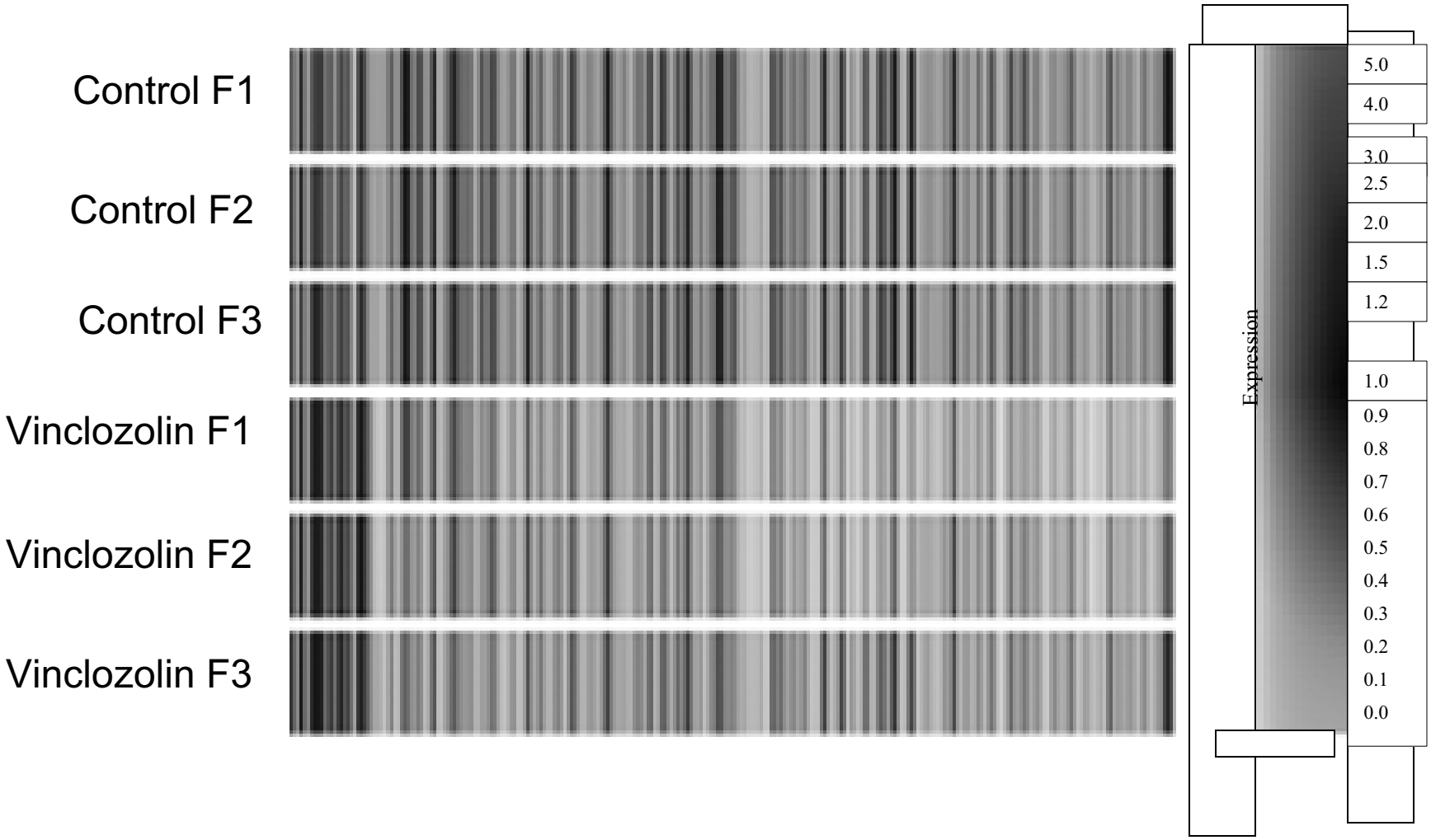
Skinner, 2005





Not just the testis affected:
Virtually all organs/systems
…for 4 generations!

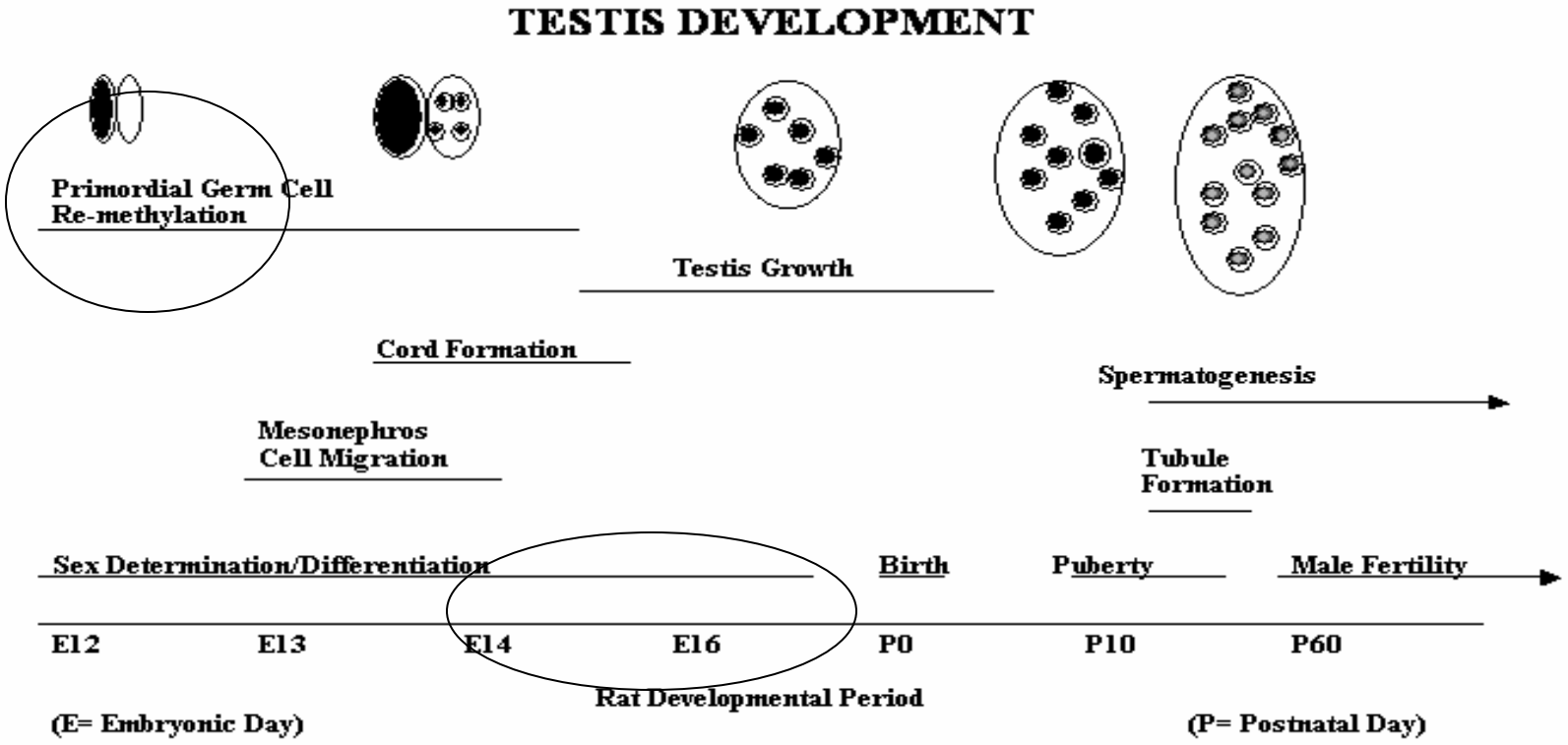
E16 Testis Transcriptome



Same results from Adults

Michael Skinner

Window of Susceptibility to Methoxychlor Transgenerational Effects



Concept

- Dosing during the time of primordial germ cell resetting of the epigenetic marks can result in germ cell transmission of a toxic effect.

- **What your great-grandfather and grandfather were exposed to can affect your health.**

Thought Provoking Idea!

Could it be that

Maternal grooming behavior

could influence adult sensitivity to stress and disease ?

Maternal Behavior in the Rat

M Szyf

Maternal Behavior in the Rat

M Szyf

Maternal behavior programs GR gene activity in the hippocampus which lasts into adulthood

GR GENE

PUP (Day 1-6)

LOW LG

HIGH LG

ADULT (Day 90)

GR GENE

GR GENE

GR RECEPTORS

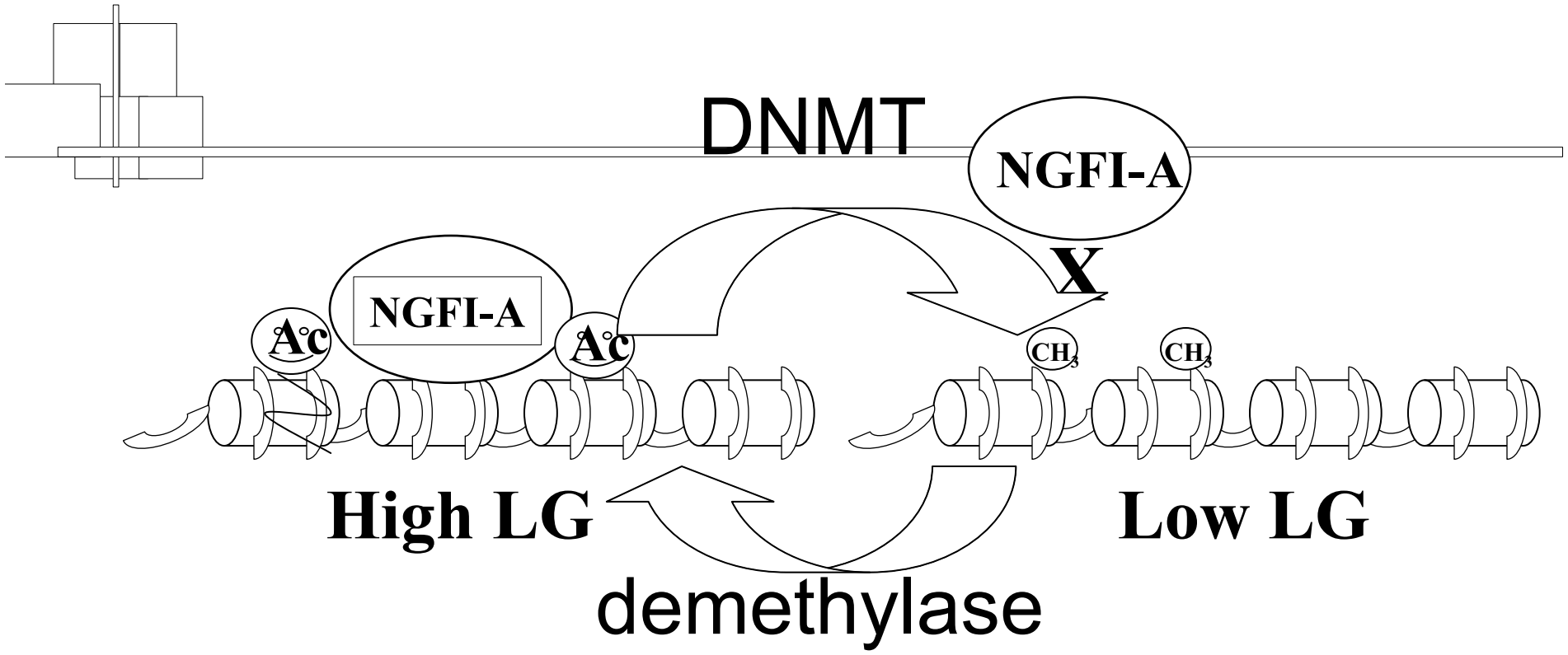
GR RECEPTORS

STRESS RESPONSE

STRESS RESPONSE

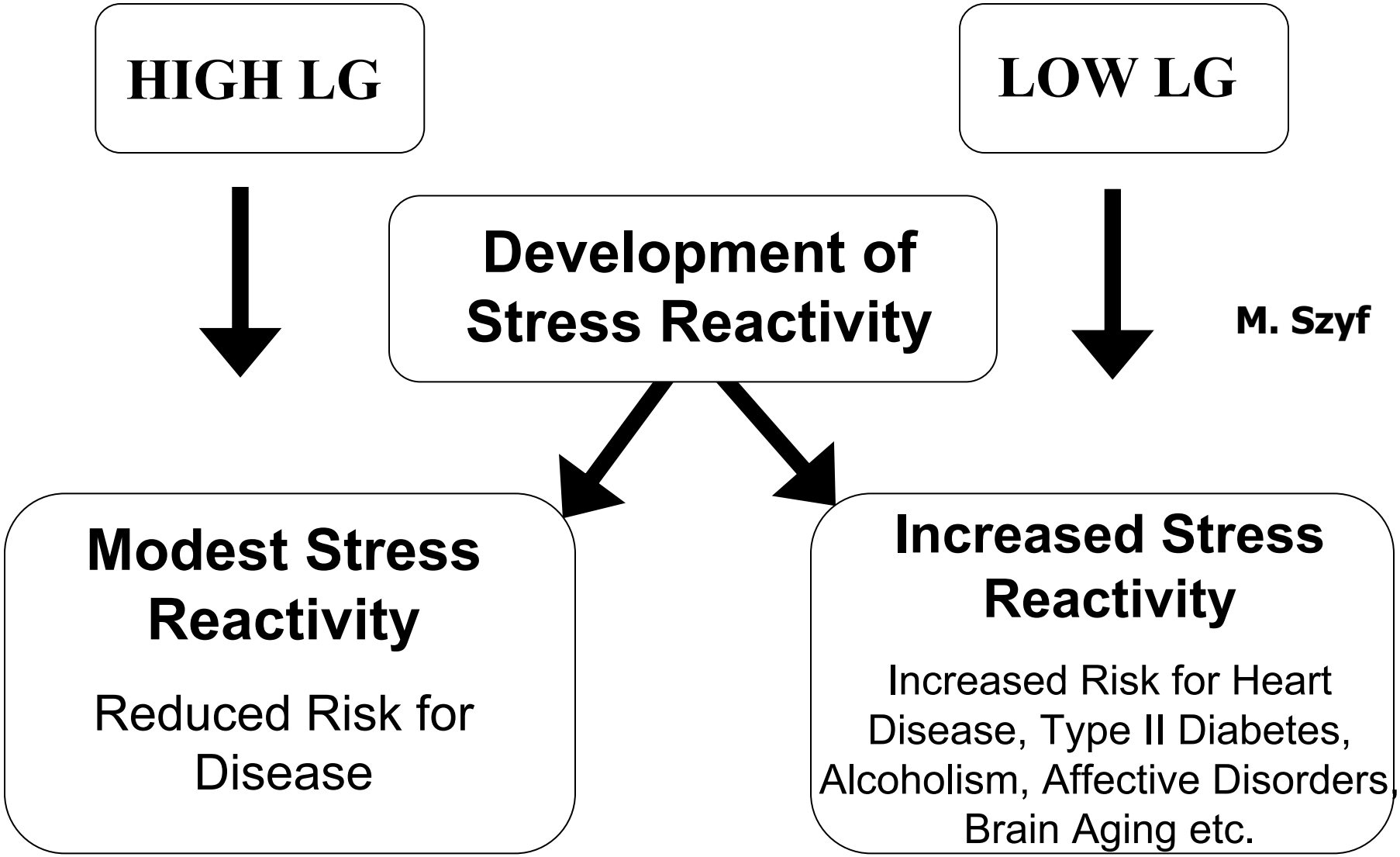
LG increases histone acetylation and binding of NGFI-A to the hippocampal GR(1_7) promoter

Methylation of CG 16 in [GR(1_7)] promoter inhibits binding of the transcription factor NGFI-A *in vitro* and *in vivo*



Weaver at al., Nature Neuroscience August 2004

Individual differences in stress reactivity of the ***adult*** *are* ***determined by maternal behavior during infancy***



Concept

- Social behavior of one subject (mother) can effect epigenetic programming in another subject (child).

- Behavior responds to the environment via epigenetics.

The Developmental Basis of Disease Changes Everything!

- Developmental nutrition and environmental chemical exposures alter gene expression, via epigenetics, leading to functional changes in tissues…leading to increased susceptibility to disease.

- This implies that health outcomes, can be determined by environmental exposures that occurred in early life, possibly decades, before disease becomes apparent.

- There are now numerous examples in animal models of the developmental basis of disease.

  Fibroids, Breast Cancer, Prostate Cancer, Fertility

  Obesity, Altered Behavior

The Developmental Basis of Disease Changes Everything!

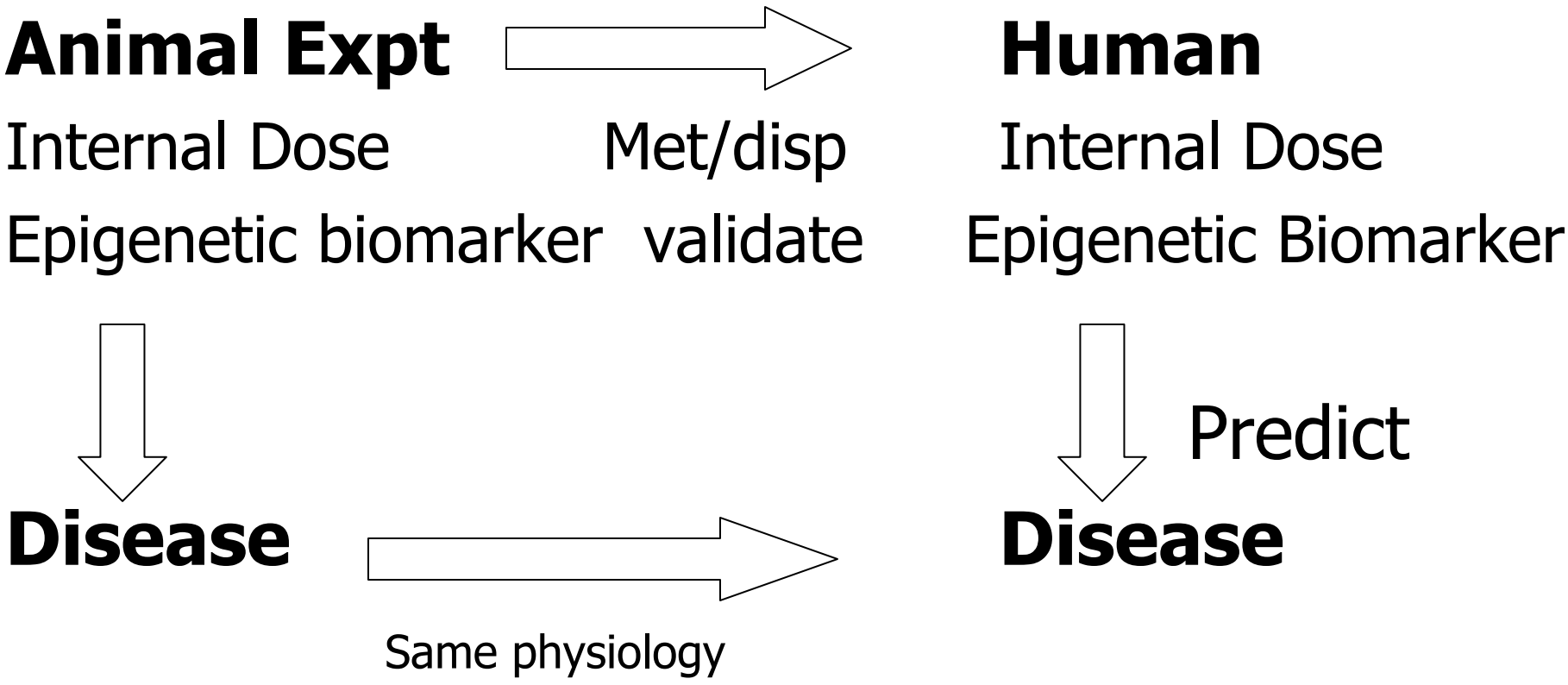
- This paradigm changes the focus from curing a disease to prevention and intervention strategies to reduce disease incidence.
- It also changes focus from adults to development for the cause of disease.
- Identification of an "imprint" left by developmental programming such as altered methyl marks may be useful for identification of exposed individuals and as a biomarker for disease susceptibility in adult life.

How to Assess Human Risk from Developmental Exposures?

- Problem 1: How to determine exposures during specific windows of exposure
- Problem 2: How to assess functional change
- Problem 3:. How to relate functional change to disease later in life
- Problem 4: How to conduct studies for 60 years.

It all comes down to the need for validated biomarkers from animal studies that can be used in humans to indicate potential increase in susceptibility to disease later in life.

Strategy for Assessing Risk



What is Needed?

- Examine more diseases
- Better animal models of disease
- Internal exposure measurements, animals and humans
- Biomarkers of exposure and effect
- Epigenetic biomarkers
- Translation of biomarkers to human studies
- Human studies (dev exposures and biomarkers of effect)
- Lifespan approach
- Mixtures
- Team science…animal/human studies
- Team science…focus on syndromes (phthalate, estrogens)
- New exposures from new sources

Thank You !